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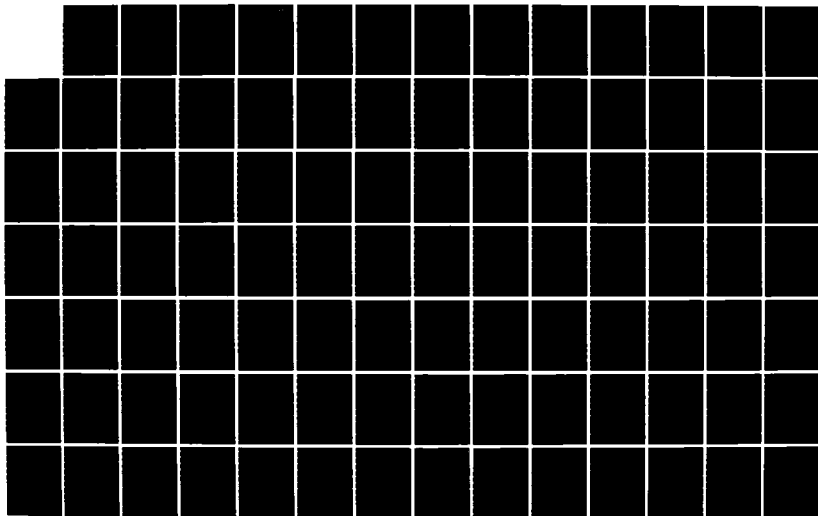
THE SUBCHRONIC INHALATION TOXICITY OF DF2 (DIESEL FUEL) 1/2  
USED IN VEHICLE E. (U) CHEMICAL RESEARCH AND  
DEVELOPMENT CENTER ABERDEEN PROVING GRO.

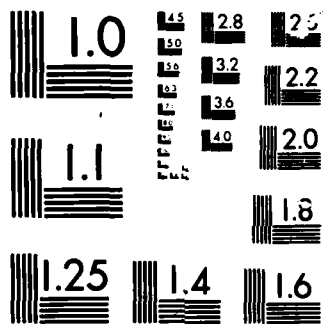
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# THE SUBCHRONIC INHALATION TOXICITY OF DF2 (DIESEL FUEL) USED IN VEHICLE ENGINE EXHAUST SMOKE SYSTEMS (VEESS)

by

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March 1986

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<p>Mice and rats were exposed by the airborne route to average total hydrocarbon concentrations of 2,340 ± 450 mg/cu m of M60A1 tank-generated DF2 smoke/exhaust or 6.0 ± 6.0 mg/cu m of DF2 exhaust only. The exposures were performed under static airflow conditions and consisted of 15- or 60-min daily exposures lasting for up to 13 weeks. Toxicological, physiological, hematological, blood chemical, behavioral, reproductive, mutagenic, teratogenic, and pathological effects were evaluated.</p> <p>Two consistent significant toxicological effects were found as a result of the exposures to either type of emission. The toxic sign hypoactivity was observed in both species after daily 60-min exposures to the DF2 smoke/exhaust mixture. The other effects were fetal toxicity and possible fetal abnormalities in some rat pups delivered from pregnant dams that had been exposed (60-min daily) to the DF2 smoke and/or exhaust emissions during the 6th to 15th day of gestation.</p> <p>(Continued on reverse)</p>				
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On the basis of these and previous findings, it is recommended that personnel wear protective masks while stationed in the midst of DF2 smoke and/or exhaust emissions generated by the M60A1 tank Vehicle Engine Exhaust Smoke System at the concentrations used in these studies. *Key words:*

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## PREFACE

The work described in this report was authorized under Project No. 1L162622A552, Technical Area 4-E, Smoke Toxicology. The work was started in August 1979 and completed in August 1981. The experimental data are contained in notebooks DSD 10,131, 10,009, 10,005, 10,096, 10,000, 10,083, 10,111, 9425, TSD 9641, and 9862; CSL 81,0018, 81,0197, and 81,0198. The chromatographic records for the total hydrocarbon concentrations, climatic parameters of the exposure chambers, gas concentrations, hematological and blood chemical values, and pathological findings for the test animals are in the archives of the Toxicology Division, Research Directorate, U.S. Army Chemical Research and Development Center, Aberdeen Proving Ground, Maryland 21010-5423.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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This report has been approved for release to the public.

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THE SUBCHRONIC INHALATION TOXICITY OF DF2 (DIESEL FUEL)  
USED IN VEHICLE ENGINE EXHAUST SMOKE SYSTEMS  
(VEESS)

1. INTRODUCTION

Toxicity studies were conducted to determine the effects of sub-chronic inhalation from multiple total body exposures to DF2 diesel fuel smoke and/or exhaust generated from the Vehicle Engine Exhaust Smoke System (VEESS) installed in an M60A1 tank. These tests were designed to produce daily exposures that would be similar to those encountered in simulated tactical exercises by unmasked personnel. The M60A1 tank and VEES were used to generate the smoke and/or exhaust. No other methods of dissemination were considered suitable to mimic the combination of the engine exhaust products and oil smoke produced by combustion/volatilization and recondensation in the engine manifold.

The fuel used in this test was diesel fuel #2 (DF2), which is used in the VEES system as a summer grade fuel. The target concentration for the generated DF2 smoke was 2.0 mg/l (2,000 mg/cu m), which is shown in Figure 1\* to be approximately 20% of the theoretical average smoke concentration predicted for exposure of personnel standing downwind within 10 meters of the tank when it is operating at maximal smoke-generating efficiency under atmospheric conditions of strong inversion and low wind speed. Because dissemination of smoke generated from the M60A1 tank also involves normal exhaust emissions from internal engine combustion, the concentration of this component was also monitored and evaluated for toxicological effects. The average total hydrocarbon concentration of the exhaust for DF2 diesel fuel was found to be about 0.30% of the smoke/exhaust concentration.

In general, two species of animals were exposed to tank-generated DF2 smoke and/or exhaust in a 20,000-liter chamber with static air flow for either 15 and 60 minutes. Toxicological, hematological, blood chemical, physiological, behavioral, mutagenic, teratogenic, reproductive, and pathological effects in exposed animals were evaluated over the 32- and 65-day exposure periods. Some animals were also evaluated 30 days after the 65-day exposure period.

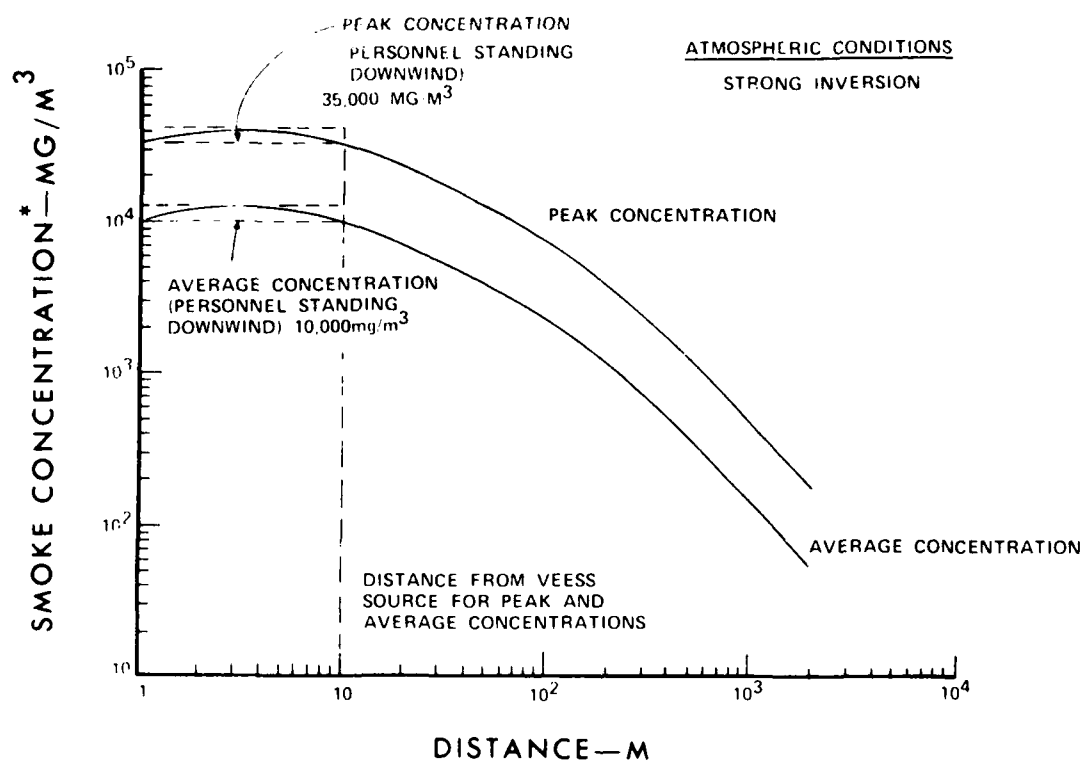
2. EXPERIMENTAL PROCEDURES

2.1 Generation of DF2 Smoke and/or Exhaust from the M60A1 Tank.

2.1.1 Physical and Chemical Composition for DF2 Diesel Fuel.

DF2 diesel fuel consists of mixtures of aliphatic and aromatic hydrocarbons obtained from the atmospheric distillation of petroleum or a blend of these with hydrocarbons obtained by breaking down compounds that boil at higher temperatures. The chemical composition of DF2 diesel fuel can vary from batch

\*DF, DRDAR-CYL-A dated 6 June 1978, Subject: Smoke Concentration Profiles For Vehicle Engine Exhaust Smoke Systems (VEES). From the Chief, Systems Assessment Office to the Chief, Munitions Division, Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland. UNCLASSIFIED.



\* DIESEL FUEL DROPLETS

Figure 1. The Optimal Capacity of a Vehicle Engine Exhaust Smoke System to Generate Diesel Fuel Smoke Operating Under Inverse Atmospheric Conditions

to batch and is controlled only by the interaction of such parameters as boiling point, cetane number, viscosity, and flash point, which vary according to environmental conditions.

The DF2 diesel fuel used in these studies was obtained from the Material Testing Directorate, U.S. Army Test and Evaluation Command. It was compounded for vehicles with high-speed diesel engines, such as tanks and trucks, and is designated for use in warm areas. On this basis its composition requires less volatile components than those fuels used in colder climates.

Table A-1 (Appendix A) lists the general requirements for DF2 diesel fuel that are listed in Federal Specification VV-F-800B. Tables A-2 and A-3 present the analyses for water and sediment content, cloud point, kinematic viscosity, flashpoint, ash content, and distillation range of the fuel samples used in these studies. Analyses were performed by the Physical Test Branch, Measurement and Analysis Division, U.S. Army Test and Evaluation Command, Aberdeen Proving Ground, Maryland.

#### 2.1.2 Filling Procedure for the Exposure Chamber.

Tank generation and dissemination of DF2 diesel fuel smoke and/or exhaust consisted of accelerating the 750-HP Chrysler engine up to 1700 rpm for approximately 15 min. During acceleration, the tank was kept in a stationary position with no load on the engine. When the engine manifold temperature reached 1180 °F, the selected fuel was injected into the manifold through a stainless-steel orifice at a rate of 1,720 ml/min.

The exposure chamber was filled with the desired concentrations by passing the generated smoke and/or exhaust from the tank manifold through a 6-inch flexible stainless steel tube attached to an aluminum plenum. The same plenum was connected to a 24- x 24-inch window in a 20,000-liter cylindrical exposure chamber whose aperture was controlled by a gate valve. The plenum also had sealed entry into a wind tunnel that provided and controlled the velocities of the air/DF2 smoke and/or exhaust emissions as they emerged from the tank.

On this basis, the mechanics of filling the chamber involved drawing the emissions through the plenum into the exposure chamber inlet. The desired concentration of DF2 smoke and/or exhaust was then attained by controlling the time of cloud introduction before closing the chamber inlet door.

In these studies the tank was allowed to generate the DF2 smoke and/or exhaust into the wind tunnel for 5 min before the chamber inlet window was opened to allow emission entry. It should also be mentioned that a port for air bleeding was located at the connection between the tank manifold's flexible hose and the wind tunnel plenum. The ratio of air to DF2 smoke and/or exhaust at this port was determined to be 20:1.

The reason for conducting the animal exposures with static airflow was that the VEES system produces extremely high smoke concentrations that are capable of overwhelming (e.g., plugging) the wind tunnel filtration system if generated continuously and dynamically. The wind tunnel filtration system consists of 210 (2-ft x 2-ft) absolute particulate filters.

Caged animals were placed in the chamber before introducing the emissions. At the end of the designated exposures, chamber valves were opened and the smoke and/or exhaust was evacuated over a 10-min period.

It should be mentioned that the DF2 smoke/exhaust cloud diminished to an average of 35% of original concentration over a 60-min period as compared to 44% for the exhaust.

The same procedure was followed for the "exhaust only" exposure, omitting expulsion of diesel fuel into the hot engine manifold.

#### 2.1.3 Measurement of Exposure Chamber Gas.

During a 60-min exposure, ambient and chamber temperatures and relative humidity were measured with a Honeywell Instrument Co. analyzer (Model #W809A) before and at 0, 1, 5, 10, 15, 30, and 60 min after smoke and/or exhaust entry into the chamber.

Carbon dioxide concentrations were measured at the same intervals as temperature and relative humidity. Oxygen content was measured at 0, 10, and 60 min. Carbon monoxide was measured once per exposure and about 10 min after emission of the DF2 smoke and/or exhaust into the chamber. In the case of 15 min exposures, similar measurements were taken, except that the final measurements were at 15 min.

Carbon dioxide was measured on a Horiba Ltd infrared analyzer, Mexa-300. The oxygen content of the chamber was obtained by measuring aliquots from grab samples on a Model E2 Beckman Analyzer. Carbon monoxide was measured with a Bendix Gastec Precision Gas Detection Tube (Cat. No. ILL) with a range of 5 to 50 ppm. The sample of carbon monoxide was drawn into the tube by means of a UNICO (Union Industrial Equipment Corp., Fall River, MA) Mod. 400 Precision Pump.

Several individual measurements for nitrogen dioxide, sulfur dioxide, and ethylene oxide were taken throughout the experiments to determine the chamber concentrations of these gases in the emissions, usually 10 and/or 40 min after smoke and/or exhaust entry. The procedure consisted of attaching a Drager (Dragerwerk-AG-Lubeck, Germany) precision gas-detector tube, sensitive to the specific gas, to a precision bore flowmeter (F&P Co., Tube No. 2-S-150113, 25-liter capacity) by means of rubber tubing 1/8 inch in diameter. The flow meter was in turn connected to a Fischer Scientific Vacuum Pump (Model No. 5KH35KG-846) operating at 1725 rpm and a vacuum pressure of 30 lb/sq inch.

The detection ranges of the Drager tubes were as follows: nitrogen dioxide (Cat. No. 6722584), 2 to 100 ppm; sulfur dioxide (Cat. No. 6728491), 0.5 to 25 ppm; and ethylene oxide (Cat. No. 6728241), 25 to 500 ppm. Nitrogen dioxide was sampled at a rate of 1 l/min for 1 min as compared to a rate of 0.2 l/min for 1 min for sulfur dioxide. Ethylene oxide was sampled at a rate of 0.75 l/min for a period of 2 to 4 min.



#### 2.1.4 Methodology for Sampling the Hydrocarbon Content of the Chamber.

Samples of DF2 smoke/exhaust were collected from the chamber with the modified Mine Safety Appliance Co. Electrostatic Precipitators (Model No. Series F) at a flow rate of 5 l/min for 2 min. Collection times were at 8 to 10, 28 to 30, and 58 to 60 min for each exposure hour. For 15-min exposures, collection times were at 8 to 10 and 13 to 15 min. After DF2 smoke/exhaust sampling, the precipitator canisters were washed with 20 ml of hexane and 2- to 5- $\mu$ l aliquots were extracted for gas chromatographic analysis.

Modified Mine Safety Appliance Co. electrostatic precipitators (Model No. Series F) were also used for chamber sampling of the total hydrocarbon content of DF2 exhaust. Sampling rate was 20 l/min for 10 min, and samples were taken with a single precipitator at 5 to 15, 25 to 35, and 50 to 60 min for each exposure hour. For 15-min exposures, DF2 exhaust samples were taken with two precipitators, positioned vertically and located approximately 14 in. from each other. With this arrangement, dual samples of the exhaust were collected simultaneously from 5 to 15 min. The precipitated hydrocarbon samples were extracted from the canisters with 15 ml of hexane. Chromatographic analyses were performed with 4- to 10- $\mu$ l aliquots of the mixture.

The gas chromatographic parameters used during the chemical analysis for the hydrocarbon content of DF2 (diesel fuel) smoke and/or exhaust are shown in Table 1. A detailed description of the methodology for sampling the total hydrocarbon content of both DF2 smoke and/or exhaust is described in Table 2.

#### 2.1.5 Particle Size Measurements (Geometric Mean Diameter).

The 20,000-liter chamber was filled with the smoke and/or exhaust to the appropriate concentrations and allowed to equilibrate for at least 5 min. The isokinetic nozzle of a Sierra (Model No. 2210K) ambient cascade impactor was inserted into a chamber port. Airflow for the impactor was maintained with a Sierra constant airflow sampler (Model No. 116-400) with a capacity of 1 to 20 lpm. The sampling rates ranged from 5.0 to 8.5 lpm for periods of 1 to 10 min.

When sampling was completed, the impactor was disassembled and the 10 metal stages, each containing (Type A) glass-slotted fibers (Sierra Model No. C-220-GF), were rinsed and/or soaked in hexane for carbonaceous particle desorption. A Reeve-Angel No. 934AH (47-mm) glass fiber filter used behind the 10th stage of the impactor was also soaked in hexane. Ten milliliters of solvent was used for rinsing and/or soaking each stage/filter assembly.

After soaking and shaking, 2-ml quantities of the effluent were placed in cuvettes and read for optical density (absorbance) on a Beckman Model No. 25 Spectrophotometer.

If carbonaceous particle concentrations were too high for absorbance (optical density range), serial dilutions were prepared. Recordings were made at a wave length of 273 nm.

Table 1. Gas Chromatographic Parameters Used in the Chemical Analysis of the Total Hydrocarbon Content of DF2 (Diesel Fuel) Smoke and/or Exhaust Generated by the M60A1 Tank

Parameter	DF2 Smoke and/or Exhaust
Temperature 1 (°C)	110
Time 1 (min)	0.50
Rate (min)	8.00
Temperature 2 (°C)	260
Time 2 (min)	0.50
Injection temperature (°C)	200
FID temperature (°C)	300
Oven maximum (°C)	300
Chart speed (min)	1.00
Flow A (ml/min)	34
Flow B (ml/min)	32
Injection size	Varied (2-10 $\mu$ l)
Attenuation	Varied
FID signal	+ B
SLP sensitivity	0.10
Area reject	8000

Table 2. Chemical Procedure for Determining the Total Hydrocarbon Content of M60A1 Tank-Generated DF2 Smoke and or Exhaust

Method	Smoke/Exhaust	Exhaust
1. Attempted airborne concentration (mg/l)	2.0	0.01
2. Sampler	Modified Mine Safety Appliance Co. Electrostatic Precipitator (Model No. Series F)	Modified Mine Safety Appliance Co. Electrostatic Precipitator (Model No. Series F)
3. Sample collection time (sec)	35	35
4. Dilution of air-to-hydrocarbon mixture	20:1	20:1
5. Sample collection flow rate (l/min)	5	20
6. Sampling time (min)	2	10
7. Sampling periods (min)	15 (8-10, 13-15) 60 (8-10, 28-30, 58-60)	15 (5-15) 60 (5-15, 25-35, 50-60)
8. Chemical analysis	Wash contents of the canister from precipitator with 20 ml of hexane. Analyze (2- to 5- $\mu$ l) aliquots on Hewlett-Packard GC (Model Nos. 5830A or 5840A). Six-in. glass column (1/4-in. OD) Pyrex (4-mm ID). Column packing - 5% or 17% on 60-80 gas chrom. P. Use Hewlett-Packard GC terminal No. 18850A.	Wash contents of the canister from the precipitator with 15 ml of hexane. Analyze (4- to 10- $\mu$ l) aliquots as described for smoke/exhaust mixture.

Calculations for total carbonaceous particle mass were made as follows:

$$\frac{\text{Optical density of 2-ml sample}}{\text{Slope factor of standard curve (156)}} \times \frac{\text{Dilution factor (ml)}}{\text{Total mass of carbonaceous particle sample per stage of impactor (ug)}} =$$

$$\text{Cumulative total mass} = \frac{\text{Summation of carbonaceous particle mass found in each filter and impactor stage including the 10th stage back-up filter}}{\text{Total mass of carbonaceous particle sample per stage of impactor (ug)}}$$

A typical example of the use of these calculations is shown in Table 3. Calculation of the geometric mean diameter, with geometric standard deviations, of carbonaceous particles collected from the DF2 (diesel fuel) smoke and/or exhaust emissions was determined on the Univac 1100 computer according to the statistical method of Marple and Rubow.<sup>1</sup> A typical example of the data from which these calculations were determined is shown in Table 3.

#### 2.1.6 Chemical Kinetics and Chemical Analysis of the Total Hydrocarbon Content of DF2 Smoke and/or Exhaust.

##### 2.1.6.1 Chemical Kinetics of the Total Hydrocarbon Content of DF2 Smoke and/or Exhaust.

The chromatograms of DF2 smoke and/or exhaust were found to have 15 to 18 distinct peaks under these conditions. Figure 2 is an example of a typical chromatogram obtained during the generation of DF2 smoke/exhaust with the M60A1 tank. The attempted total hydrocarbon concentration shown by this example was 2000 mg/cu m, the target DF2 smoke/exhaust concentration sought in these studies. The target concentration for DF2 exhaust in these tests was 10.0 mg/cu m.

Regardless of the fact that the DF2 smoke/exhaust emissions were a combination of volatilized diesel fuel droplets and hydrocarbon and/or carbonaceous by-products from internal combustion and that the exhaust was a mixture of hydrocarbon and/or carbonaceous by-products from internal combustion only, the resulting hydrocarbon peaks, retention times, and yield calculations were

Table 3. The Mass Distribution of Carbonaceous Particles Collected From a Diesel Fuel (DF2) Generated Smoke/Exhaust Cloud Disseminated in an Animal Exposure Chamber

28 October 1979

Impactor stage no.	Particle size (microns)	Optical density (absorbance) <sup>a</sup>	Dilution (ml)	Sample concentration (µg/ml)	Total mass (micrograms)	% of Total mass	Cumulative % of total mass
Filter	---	0.304	10.0	47.4	474.0	4.0	4.0
10	---	0.358	10.0	55.8	558.0	4.7	8.7
9	0.10	1.015	10.0	158.3	1583.0	13.2	21.9
8	0.29	2.850	10.0	444.6	4446.0	37.1	59.0
7	0.47	0.980	10.0	152.9	1529.0	12.8	71.8
6	0.85	0.438	10.0	68.3	683.0	5.7	77.5
5	1.50	0.388	10.0	60.5	605.0	5.1	82.6
4	2.40	0.350	10.0	54.6	546.0	4.5	87.1
3	4.0	0.387	10.0	60.4	604.0	5.0	92.1
2	10.0	0.360	10.0	56.2	562.0	4.6	96.7
1	16.0	0.250	10.0	39.0	390.0	3.3	100.0

Sample collection rate 8.5 liters/minute for 15.0 minutes

Time of sample collection after formation of cloud  
 $\frac{\text{Time} = 10 - 18.5 \text{ minutes}}{\text{Chamber temperature} = 71.0 \text{ }^{\circ}\text{F}}$   
 $\frac{\text{Chamber relative humidity}}{\text{humidity}} = 53.0\%$

Total Mass (µg) 11,980  
 $\frac{11,980}{127.5} = 94.0 \text{ } \mu\text{g/liter (Cascade Impactor Collection)}^b$   
 Total Liters Collected 127.5

<sup>a</sup>Beckman-Model 25 Spectrophotometer - wave length = 273 nm  
<sup>b</sup>Sierra-Model 2210K - 10 stage ambient cascade impactor

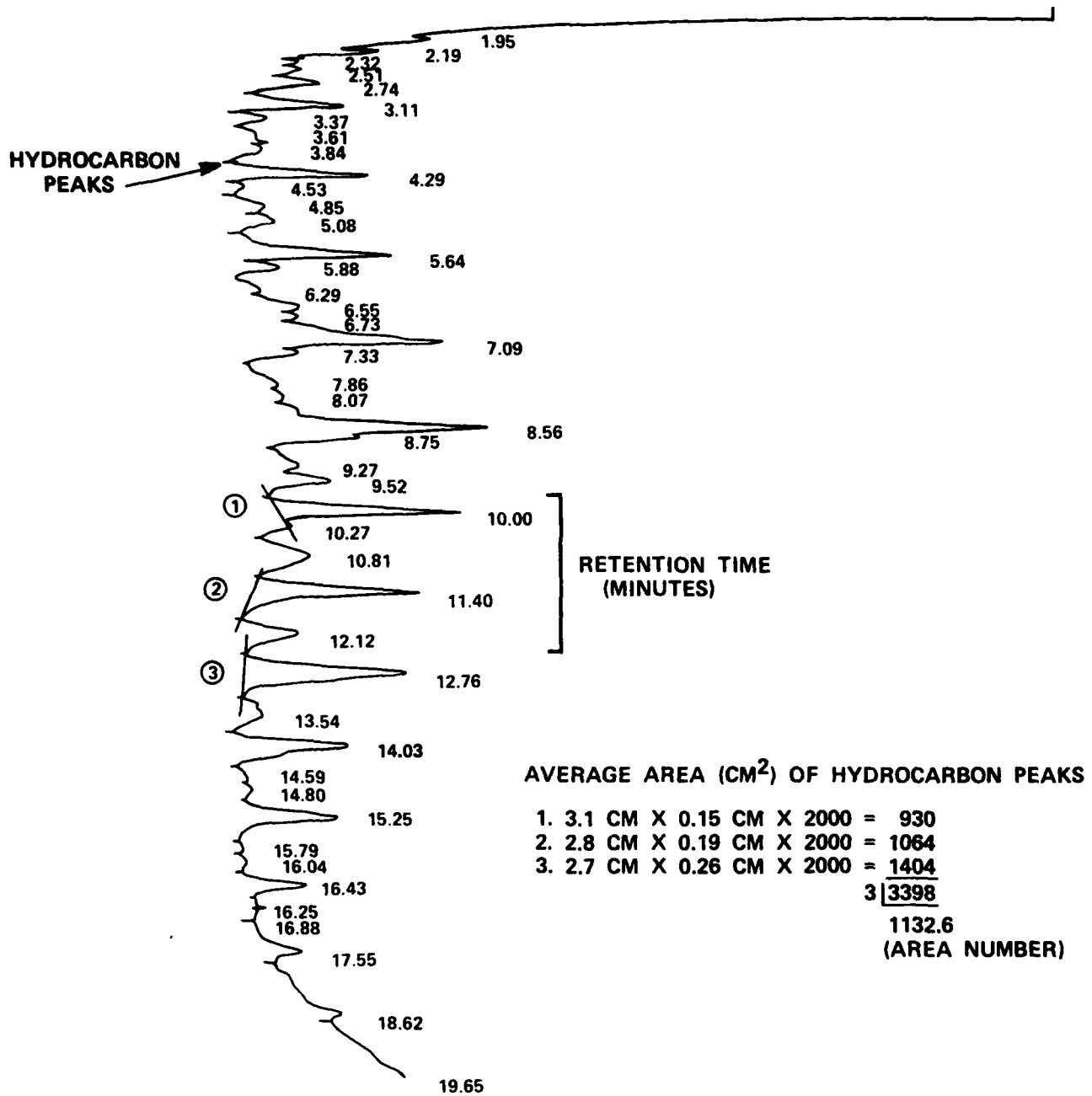


Figure 2. A Typical Chromatogram of the Hydrocarbon Peaks Derived From a DF2 (Diesel Fuel) Smoke/Exhaust Cloud Generated by the M60A1 Tank

similar. Three of the major peaks were used for quantitating the concentrations of DF2 smoke and/or exhaust. The retention times for these peaks was:

Diesel Fuel	Type of Cloud	Retention Times (min)
DF2	Smoke/exhaust	10.00-10.10
		11.10-11.45
		12.75-12.80
DF2	Exhaust	11.80-11.90
		13.30-13.40
		14.80-14.90

The neat and dilute DF2 diesel fuel standards in hexane were found to have comparable spectra. The composition of DF2 diesel fuel did not undergo any significant change at temperatures up to 650°C during laboratory smoke generation. The retention times of the major peaks were not affected by any of the extracted filters or collection columns from the original diesel fuels.

#### 2.1.6.2 Chemical Analysis for the Total Hydrocarbon Content of DF2 Smoke and/or Exhaust.

Analyses were performed using Hewlett-Packard Gas Chromatograph Models 5830A and 5840A under the following conditions:

<u>Column</u>	- Pyrex glass, 6-ft, 1/4-in. OD; 4-mm ID
<u>Column Packing</u>	- 5% OV-17 on 60-80 gas chrom. P.
<u>Oven</u>	- 60-180 °C at 4°/minute 180-240 °C at 15°/minute
<u>Inlet</u>	- 280 °C
<u>Carrier</u>	- Nitrogen (prepurified)
<u>Detector</u>	- (FID)
<u>Ionization</u>	- 270 °C

All parameters measured during the gas chromatographic analyses are shown in Table 1. A Hewlett-Packard terminal, Model #18850A, was used to set and record the GC parameters, record the chromatogram, and measure the area under each peak on the chromatogram. Solutions (2- to 10- $\mu$ l aliquots) of stock DF2 diesel fuel in hexane, prepared daily in the same hydrocarbon concentration ranges as the M60A1 tank-generated smoke and/or exhaust, served as baselines for the analyses.

The following example using DF2 diesel fuel for a 1-hr smoke/exhaust test (attempted smoke/exhaust concentration = 2.0 mg/l) demonstrates the method used for determining the concentrations of DF2 diesel fuel smoke and/or exhaust in the exposure chamber.

- A standard solution was prepared by adding stock DF2 diesel fuel to hexane, with a concentration of 1.093 mg/ml as the target.
- A gas chromatogram of the standard was acquired using 5- $\mu$ l aliquots of the standard solution. The area of the standard was obtained from the peaks at retention times of 10.00, 11.40, and 12.76 min using the following formula:

$$\text{Area} = \text{height} \times \text{width of peak at half height} \times 2000$$

(where 2000 is a concentration adjustment factor obtained by comparing the magnitudes of gas chromatographic and fluorometric measurements of diesel fuels) and averaged during determination of the areas of the three peaks involved:

Hydrocarbon peak retention time (min)	Height (cm)	Width of peak at half height (cm)	Area (sq cm)
10.00	3.1	0.15	$\times 2000 = 930.0$
11.40	2.8	0.19	$\times 2000 = 1064.0$
12.76	2.7	0.26	$\times 2000 = 1404.0$
Average area of standard DF2 (diesel fuel solution)			$= 3 \quad 3398.0$
			$= 1132.6$

- A 5- $\mu$ l aliquot of a mixture of DF2 smoke/exhaust in hexane was chromatographed, and the area of each peak and the average area of the chamber's sample were obtained in the manner described above. The chamber sample of DF2 smoke/exhaust was collected with a modified Mine Safety Appliance Co. Electrostatic



Precipitator (Model No. Series F), 8 to 10 min after emission entry into the animal exposure chamber. The results of the measurements are:

Hydrocarbon peak retention time (min)	Height (cm)	Width of peak at half height (cm)	area (sq m)
10.04	5.4	0.15	x 2000 = 1620.0
11.43	5.25	0.18	x 2000 = 1890.0
12.79	5.0	0.25	x 2000 = 2500.0
Average area of chamber hydrocarbon sample collected between 8 to 10 min			3   6010.0 = 2003.3

• The concentration of the chamber sample in mg/l that was collected between 8 to 10 min was determined as follows:

Hydrocarbon peak area of chamber sample (sq m)	x	Concentration of standard (mg/ml)	x	Volume of hexane used to dilute or extract chamber hydrocarbon sample (ml)	Total hydrocarbon concentration of DF2 smoke/exhaust in chamber sample (mg/l)
Hydrocarbon peak area of standard (sq m)	x	Volume of air containing hydrocarbon sampled (l)			

$$\frac{2003.3 \text{ sq m} \times 1.093 \text{ mg/ml} \times 20.0 \text{ ml}}{1132.6 \text{ sq cm} \times 10 \text{ l}} = 3.866 \text{ mg/l (or 3,866 mg/cu m)}$$

• Chromatograms of samples of DF2 smoke/exhaust collected 28 to 30 and 58 to 60 min after entry of the cloud into the chamber were also obtained. Five-microliter aliquots of the chamber samples in hexane were used. The area of each peak, the average area of the chamber samples, and the concentration in mg/l were obtained by the method described previously.

28 to 30 Minute Sample

Hydrocarbon peak retention time (min)	Height (cm)	Width of peak at half height (cm)	area (sq m)
10.01	2.10	0.15	x 2000 = 630.0
11.41	2.30	0.18	x 2000 = 828.0
12.77	2.35	0.29	x 2000 = <u>1363.0</u>

Average area of chamber hydrocarbon sample collected (28 to 30 min)  $3 \frac{2821.0}{3} = 940.3$

Total hydrocarbon concentration of DF2 smoke/exhaust sample =

$$\frac{940.3 \text{ sq cm} \times 1.093 \text{ mg/l} \times 20 \text{ ml}}{1131.6 \text{ sq cm}^2 \times 10^{-1}} = 1.814 \text{ mg/l or } 1,814 \text{ mg/cu m}$$

58 to 60 Minute Sample

Hydrocarbon peak retention time (min)	Height (cm)	Width of peak at half height (cm)	Area (sq cm)
10.01	0.65	0.15	x 2000 = 195.0
11.41	0.80	0.20	x 2000 = 320.0
12.77	0.90	0.25	x 2000 = <u>450.0</u>

Average area of chamber hydrocarbon sample collected (58 to 60 min)  $3 \frac{965.0}{3} = 321.6$

Total hydrocarbon concentration of DF2 smoke/exhaust sample =

$$\frac{321.6 \text{ sq cm} \times 1.093 \text{ mg/l} \times 20 \text{ ml}}{1132.6 \text{ sq cm}^2 \times 10^{-1}} = 0.620 \text{ mg/l or } 620 \text{ mg/cu m}$$

● The total concentrations of hydrocarbon from DF2 smoke/ exhaust obtained from the exposure chamber at the three collection periods were averaged to obtain the mean total chamber concentration for the entire hour.

Example:	<u>Time of DF2 Smoke/Exhaust Sample Collection (min)</u>	<u>Total Hydrocarbon Concentration (mg/l)</u>
	8 to 10	3.866
	28 to 30	1.814
	58 to 60	<u>0.620</u>

Average total hydrocarbon concentration of DF2 smoke/exhaust chamber cloud over a 1-hr period = 3 6.300

2.100 mg/l or  
2,100 mg/cu m

• Similar procedures were used for determining the concentrations of DF2 exhaust.

## 2.2 Studies of Inhalation Toxicity.

### 2.2.1 Experimental Design.

Following the quarantine period recommended by the Veterinary Resources Branch, USAMRICD, and certification of the test animals' health, the B6C3F1 mice and Fischer 344 rats were divided into five exposure groups. One group was designated to serve as 60-min controls exposed only to air; a second and third group were to be exposed daily (5 days per week) for 15 or 60 min to M60A1 tank-generated DF2 exhaust; and a fourth and fifth group were to be exposed daily (5 days per week) for 15 or 60 min to M60A1 tank-generated DF2 smoke/exhaust. A total of 12 mice (6 males and 6 females) and 24 rats (12 males and 12 females) were evaluated from each exposure condition at each sacrifice interval, which occurred after 6 weeks (32 exposure days), 13 weeks (65 exposure days), and 4 weeks (30 days) after 65 days of exposure. A total of 180 mice and 360 rats were used in the study.

More rats were used so that half from each exposure group could be submitted at the above intervals for pathological evaluation directly, and half could be evaluated for physiological and/or behavioral changes. A sample schedule appears in Table 4.

### 2.2.2 Animal Species and Weight.

Two species of animals were exposed in this study: B6C3F1 mice and Fischer 344 rats. A total of 180 mice bred by the Charles River Laboratory, Wilmington, Massachusetts, were used. They averaged  $24.7 \pm 2.3$  gm (males) and  $21.6 \pm 1.5$  gm (females) in weight and were approximately 6 to 7 weeks old at the time of initial testing.

The Fischer 344 rats, also bred by the Charles River Laboratory, were divided into two groups according to weight and age. An older group of 300 animals averaged  $358.0 \pm 28.0$  gm (males) and  $198.0 \pm 13.06$  gm (females) in weight and were approximately 13 weeks old at the time of initial testing.

Table 4. Schedule for Evaluation of Animals Exposed Subchronically by the Airborne Route Under Static Airflow Conditions to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Species	Total no. animals	Pathological and hematological effects (32nd day of exposure)	Pathological, hematological and blood chemistry effects	Physiological and behavioral Effects
B6C3F1 Mice	6M/6Fa 6M/6Fb 6M/6Fc 6M/6Fd 6M/6Fe	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	----- ----- ----- ----- -----	----- ----- ----- ----- -----
Fischer 344 Rats	12M/12Fa 12M/12Fb 12M/12Fc 12M/12Fd 12M/12Fe	----- ----- ----- ----- -----	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F
(65th day of exposure)				
B6C3F1 Mice	6M/6Fa 6M/6Fb 6M/6Fc 6M/6Fd 6M/6Fe	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	----- ----- ----- ----- -----	----- ----- ----- ----- -----
Fischer 344 Rats	12M/12Fa 12M/12Fb 12M/12Fc 12M/12Fd 12M/12Fe	----- ----- ----- ----- -----	6M/6F* 6M/6F* 6M/6F* 6M/6F* 6M/6F*	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F
(30 days post exposure)				
B6C3F1 Mice	6M/6Fa 6M/6Fb 6M/6Fc 6M/6Fd 6M/6Fe	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	----- ----- ----- ----- -----	----- ----- ----- ----- -----
Fischer 344 Rats	12M/12Fa 12M/12Fb 12M/12Fc 12M/12Fd 12M/12Fe	----- ----- ----- ----- -----	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F

<sup>a</sup>Controls - Exposed (60 min daily) to air

<sup>b</sup>Exposed (15 min daily) to average total hydrocarbon concentrations (6.0 + 6.0 mg/cu m) of DF2 exhaust

<sup>c</sup>Exposed (60 min daily) to average total hydrocarbon concentrations (6.0 + 6.0 mg/cu m) of DF2 exhaust

<sup>d</sup>Exposed (15 min daily) to average total hydrocarbon concentrations (2,340 + 450 mg/cu m) of DF2 smoke/exhaust

<sup>e</sup>Exposed (60 min daily) to average total hydrocarbon concentrations (2,340 + 450 mg/cu m) of DF2 smoke/exhaust

\*In some cases more than 12 rats were sacrificed for histopathological evaluation to correlate pathological and physiological findings from the same animal.

A younger group of 60 animals averaged  $192.0 \pm 17.0$  gm (males) and  $129.0 \pm 6.0$  gm (females) in weight and were approximately 10 weeks old when the tests started.

Edgewood-bred Sprague Dawley rats were used to study teratological and reproductive effects because of the extensive background information collected in this strain over the past 20 years.

#### 2.2.3 Animal Observation.

Before initiation of sequential airborne exposures to DF2 smoke and/or exhaust, all animals were ear-tagged, assigned randomly to groups, and weighed. Thereafter, they were weighed weekly during the 65-day exposure and 30-day post-exposure periods. In addition, animals were preselected from each group to be observed for toxic signs immediately after the daily exposure, within 24 hr later, and once daily during the 30-day post-exposure period.

During exposure in the chamber, the mice were placed individually in stainless-steel cages 20-in. L x 14 in. W x 3-in. H that contained 10 compartments that were 7-in. L x 4-in. W x 3-in. H. Each cage was surfaced with multi-mesh openings (1/2-in. x 1/2-in.) for uniform ventilation and exposure. The rats were treated similarly except that their cages measured 30.5-in. L x 18.0-in. W x 5.5-in. H with 9-in. L x 6-in. W x 5.5-in. H compartments. These cages were also surfaced with 1/2-in. x 1/2-in. mesh openings. During exposure, the animals were not given food, water, or bedding.

After exposure, the animals were placed in plastic cages in groups of three and given certified Purina Rodent Chow and Water ad libitum. Control and exposed animals were housed separately in a climatically controlled "bioclean" room in Bldg E-3266.

#### 2.2.4 Hematological and Blood Chemical Evaluations.

B6C3F1 mice and Fischer 344 rats scheduled for necropsy were also bled for hematological and/or blood chemical evaluation. Usually 12 animals (6 males, 6 females) of each species were sampled per exposure condition at each sacrifice period. The mice were evaluated for hematological changes only, while the rats were evaluated for both hematological and blood chemical effects.

For blood sampling, the animals were anesthetized intraperitoneally with sodium pentobarbital and bled by cardiac puncture. Immediately after blood sampling, they were necropsied and prepared for pathological examination. The blood samples from mice were analyzed for hematological effects only: red blood cell count, white blood cell count, differential white blood cell count, hematocrit, and hemoglobin. The blood samples from rats were analyzed for red blood cell count, white blood cell count, differential white blood cell count, hematocrit, hemoglobin, triglycerides, cholesterol, glucose, blood urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, carbon dioxide, alkaline phosphatase, total protein, albumin, globulin, calcium, phosphorus, serum glutamic pyruvic transaminase and oxalacetic transaminase, lactic dehydrogenase, and total bilirubin.

All blood samples were prepared for analysis under the standard operating procedures and conditions established by National Health Laboratories Inc. of Arlington, VA; they were analyzed by the same laboratory.

### 2.2.5 Chemistry of Blood Gases.

Four male and three female rats were exposed to 1.72 mg/l (1,720 mg/cu m) of DF2 smoke/exhaust for 60 min. The same number of animals of each sex were also exposed to 0.0052 mg/l (5.20 mg/cu m) of DF2 exhaust for the same time period. Three male and three female animals served as common controls for both exposures.

Immediately after removal from the chamber, each animal was anesthetized intraperitoneally with 35.0 mg/kg of sodium pentothal. Blood samples (3.0 to 5.0 ml) were then taken by cardiac puncture and stored in an ice bath for analyses of carboxyhemoglobin levels according to the Dithionite Reduction method.<sup>2</sup>

### 2.2.6 Physiological Evaluations.

#### 2.2.6.1 Pulmonary Responses in Rats Exposed to DF2 Smoke and/or Exhaust.

Fischer 344 rats, six males and six females from each exposure condition and period, were evaluated for pulmonary changes according to the methodology described by Cummings *et al.*<sup>3</sup> Pulmonary measurements included respiratory rate, estimated pulmonary resistance, minute volume, and tidal volume.

Due to technical difficulties, the minute volume and tidal volume were not measured in the animals in the 30-day post-exposure group. Also due to technical difficulties, data from some of the animals in the other groups was eliminated in the final statistical evaluation. The pulmonary measurements were analyzed using the Student's "t" test of statistical analysis.

#### 2.2.6.2 General Physiological Evaluation of Rats After Exposure to DF2 Smoke and/or Exhaust.

The general physiological condition of Fischer 344 rats was evaluated as previously cited. The experimental design is shown in Table 5.

Table 5. Experimental Design for Physiological Functional Tests Designed to Determine the Effects of Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> on Fischer 344 Rats.

Time	Control	Exhaust 15 min	Smoke/Exhaust 15 min	Exposure condition	
				Exhaust 60 min	Smoke/Exhaust 60 min
32 days	12	12	12	12	12
65 days	12	12	12	12	12
30 days post exposure	12	12	12	12	12

<sup>a</sup> Average total hydrocarbon concentration of M60A1 tank-generated DF2 (diesel fuel) smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m).

<sup>b</sup> Average total hydrocarbon concentration of M60A1 tank-generated DF2 (diesel fuel) exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Immediately after the exposure, the animals were taken to the laboratory to be weighed; they were then returned to their home cages and allowed to acclimatize overnight. For the next 2 days they were examined and tested according to the methods of Cummings et al.<sup>3</sup> Rectal temperature, EKG, heart rate, blood pressure, pulmonary ventilation, physical performance, reflex activity, and pulmonary function were measured. The method for evaluating pulmonary function was described earlier in this report.

The EKG's of the rats were analyzed for wave amplitudes, intervals, duration, rhythm, and axis. Pulmonary ventilation was analyzed for frequency and volume and changes in these characteristics as a response to breathing 6% carbon dioxide. All measurements were performed on unanesthetized and comfortably restrained animals.

The data were compared statistically using an Analysis of Variance (ANOVA) that distinguished differences according to sex, exposure level, and the effect of dose within sexes. Whenever a difference was found, the data were analyzed using the Student's "t" test to determine if the source of variation was significant. The logic for determining a physiological effect from the DF2 (diesel fuel) smoke and/or exhaust exposures required that the following conditions apply:

- A significance at the  $P = < 0.05$  level must be evidenced by the ANOVA and also by the "t" test between the control and an experimental condition.
- The differences must be dose related and directional so that significant differences occurring in a lower dose group, which were not reinforced by similar effects in the high dose group, would be considered a chance occurrence.
- Any differences should be time related unless an adaptive response had developed. In the latter case, the statistical judgments would reflect this possibility, particularly where other evidence was involved (e.g., pathological structural change).

#### 2.2.7 Behavioral Effects in Rats After Airborne Exposure to DF2 Smoke and/or Exhaust.

Fischer 344 rats were also evaluated for behavioral responsiveness. The tests included the Spontaneous Activity Test (SAT) and the Passive Avoidance Test (PAT).<sup>3</sup>

Twelve animals (6 males, 6 females) per exposure group were usually evaluated at each testing period. The rats were deprived of food for 24 hr before testing, but water was supplied ad libitum. Animals were evaluated four at a time in test sessions lasting 24 min. The SAT was performed first, followed by the PAT. The ensuing data was evaluated by calculating test means with standard errors. Appropriate ANOVA were also used to analyze the findings.

## 2.2.8 Teratogenic, Mutagenic, and Reproductive Studies.

### 2.2.8.1 Toxicity and Mutagenicity in *Drosophila melanogaster* (Fruit Fly).

The test for sex-linked recessive lethal mutations in *Drosophila melanogaster* (fruit fly) provides a short-term "in vivo" mutagen screening for multicellular eukaryotic organisms. The test measures the frequency of lethal mutations in approximately 20% of the total genome of the fly. Two methods were used to determine the effects of DF2 diesel fuel and M60A1 tank-generated airborne DF2 (diesel fuel) smoke/exhaust on toxicity and mutagenicity in the fruit fly.

In the first method, adult flies were fed various concentrations of liquid diesel fuel (0.1, 1.0 and 10.0%) dispersed in 1.5 ml of ethanol, diluted to 15 ml with distilled water, and added to 5.0 gm of dry food medium (Caroline Biological Formula 4-24). Wild-type Oregon-K strain red-eyed male organisms, 1 to 2 days old were used, and the food and flies were retained in plastic vials during the exposure. Light, temperature, and humidity were controlled in an incubator during the 72 hr exposure period. Fresh test medium was made daily and placed in the vials. Other flies served as negative controls by undergoing exposure to distilled water, or distilled water mixed with ethanol. Still other flies served as positive controls through exposure to methylmethane sulfonate (MMS), a known mutagen.

In the second method, wild type, Oregon-K strain red-eyed male fruit flies (1 to 2 days old) were exposed in a 20,000-liter animal exposure chamber for periods of 1 hr, four to five times daily to M60A1 tank-generated DF2 (diesel fuel) smoke/exhaust (average total hydrocarbon concentration =  $2.340 \pm 0.0450$  mg/l). One group was retained in a glass tube (35 x 200 mm) capped with a 33-mm diameter screen and exposed five times daily. Another group of the same type species was held in a wire-screened cylinder (22 x 160 mm) and exposed four times daily. All flies were observed at 24 hr after the final exposure to determine mortality.

All organisms surviving the oral and inhalation exposures were held for mutagenic testing by the Muller-5 technique.<sup>4</sup> The method involved mating exposed red-eyed male flies to paired (P1) virgin Muller-5 bar-eyed females in 7-ml test tubes containing food and water. After 7 days, the adults were discarded and the emerging offspring (F1) were backcrossed by pairs in test tubes for another 7 days. After this second mating, the adults were discarded and the emerging F2 generation was examined for the absence of wild-type red-eyed males, an indication of mutation. The scoring method was as follows:

- Tubes containing any wild type red-eyed males were scored as negative, indicating no mutation.
- Tubes containing 20 or more flies but no wild type red-eyed males were scored as a positive mutation.
- Tubes containing less than 20 flies and no wild type red-eyed males were scored as questionable and checked by back-crossing to the F3 generation for retesting.



2.2.8.2 Teratogenic, Mutagenic, and Reproductive Effects in Sprague Dawley Rats Exposed by the Airborne Route to DF2 Smoke and/or Exhaust.

These studies were conducted in accordance with the recommendations of the U.S. Environmental Protection Agency.<sup>5</sup>

All exposures were scheduled to cover specific events in the reproductive cycle of the rat. Male rats were exposed for 10 wk and evaluated for dominant lethal mutations and also for reproductive effects in the single-generation test. This exposure covered one complete cycle of spermatogenesis before the males were mated to unexposed 12-wk-old virgins (dominant lethal mutation) or 15-wk-old virgins (single generation test) that had been exposed for the last 3 wk of the males' exposure period (4 to 5 estrus cycles). The tests for teratological effects required exposure of pregnant females from day 6 to day 15 of gestation, the period of organogenesis.

During long-term exposure, animals are sometimes lost through spontaneous deaths or accidents. Therefore, to ensure an adequate number, 2 to 4 extra animals were included in each exposure group. Excess animals were randomly discarded at the end of the exposure period.

Groups for each test of reproductive effects were exposed under one of the following conditions: Noise (tank) controls, 15- or 60-min DF2 exhaust emissions, or 15- or 60-min DF2 smoke/exhaust emissions. The mean total hydrocarbon for the DF2 smoke/exhaust was  $2,340 \pm 450$  mg/cu m and for the exhaust  $6.0 \pm 6.0$  mg/cu m.

In a study of fetal toxicity and teratogenicity, 150 12-week-old virgin females were mated, two females to each male, to seventy-five 12-week old males. The females were checked for insemination every morning, which was determined by the presence of sperm in vaginal washings. Physiological saline was used as the wash fluid. The day on which sperm was found in the washing was considered day zero of gestation.

Females found to have sperm in their vaginal washings were assigned, two at a time, to either the control group or an exposure group, DF2 smoke/exhaust or DF2 exhaust, until there were 22 sperm-positive females in the 60-min control (tank noise), the 60-min DF2 exhaust, and the 60-min DF2 smoke/exhaust groups. The male rats from these matings were euthanatized and discarded.

The sperm-positive females were exposed on the 6th through 15th days (by calculation) of gestation. On day 20 of gestation, 20 dams each in the control, DF2 exhaust, and DF2 smoke/exhaust groups were euthanatized. Each dam was weighed and a laparotomy was performed to expose the uterus. The viable and nonviable implants were counted, noting the positions of nonviable fetuses. The fetuses were delivered by caesarean section, grossly examined for abnormalities, sexed, weighed, and tagged. One-half of each litter was placed in Bouin's solution for subsequent serial sectioning using the method of Wilson<sup>6</sup> for examination of the viscera; the other half was placed in 95% ethanol to harden for subsequent staining and examination of the skeletal systems. The initial data recorded were the total number of implantation sites in each

uterine horn, the number of viable fetuses, the number of nonviable fetuses (resorption sites), and any gross abnormalities. More detailed data on each pup was recorded during visceral or skeletal examination.

In a dominant lethal mutation screening, 12 proven males were randomly assigned to each of the 5 groups. Each group was exposed 5 days per week for 10 weeks. During the week after exposure, each of 10 randomly selected males in each group was housed for 5 days with two 12-week old virgin females for mating. After 5 days these females were removed and the males rested for 2 days. A second pair of virgin females was introduced for the second post-exposure mating and also removed after 5 days. The females were euthanatized 11 days after their respective separations from the males and necropsied to ascertain pregnancy and to record numbers of viable fetuses, nonviable fetuses, and corpora lutea. Data in these categories were analyzed using the Student's "t" test, the Freeman-Tukey Arc Sine Transformation followed by Student's "t" test, and the Chi square, respectively.<sup>7</sup>

To study reproduction in a single generation, groups of 12 proven male rats were exposed under each test condition for 10 weeks. Groups of twenty-four 12-week-old virgin female rats were similarly exposed for 3 weeks, a period of four to five estrus cycles. Their exposures coincided with the last 3 exposure weeks of the males. In the 11th week, the animals were caged, two females per male, for mating. Daily exposure of the females was continued through to the weaning of their neonates (the neonates were not exposed).

Twenty-four hours after birth, each pup was examined, sexed, and weighed. Each pup was reexamined and weighed 4 days after birth, at which time, in order to equalize the nursing burden on each dam, each litter was reduced to no more than 10 pups per litter. After weighing on day 21, two pups of each sex were randomly selected from each litter, euthanatized, and examined for gross external and visceral abnormalities. If none were found, the remaining pups were assumed to be normal, and they were euthanatized, and discarded. If abnormalities were found, among the first four, then each remaining pup in the litter was euthanatized and examined to determine the frequency of the abnormalities within the litter. The data were analyzed using the Student's "t" test.

#### 2.2.9 Gross and Histopathological Findings in Mice and Rats After Airborne Exposure to DF2 Smoke and/or Exhaust.

Twelve mice and rats (6 males, 6 females) from each exposure were sacrificed and examined for pathological changes. In some instances, however, more than 12 animals were necropsied to compare pathological and physiological findings on the same animals. The results are shown in Appendix Tables B-1 to B-24.

The following body organs were examined grossly and microscopically: heart, lung, liver, spleen, kidney, brain, eye, trachea, nasal turbinates, adrenal glands, stomach, urinary bladder, pancreas, thyroid, esophagus, duodenum, colon, lymph nodes, thymus, testes, epididymus, ovary, uterus, bone marrow, and skin. Total body weight, in addition to individual weights of the heart, lungs, liver, kidneys, and gonad, was also recorded for those animals that were held 30 days after the 65th exposure to DF2 smoke and/or exhaust. These measurements were used to determine possible effects on organ-to-body weight ratios as a result of the exposures.

### 3. RESULTS

#### 3.1 Exposure Chamber Conditions.

##### 3.1.1 Total Hydrocarbon Concentrations and Climatic Conditions in the Chamber During Exposures.

The DF2 smoke/exhaust cloud was dense and acrid in odor. Airborne concentrations of the mixture were based on total hydrocarbon concentration. Over the entire 17-week exposure period, average weekly total hydrocarbon concentrations ranged from  $1.45 \pm 0.240$  mg/l ( $1,450 \pm 240$  mg/cu m) to  $3.24 \pm 0.930$  mg/l ( $3,240 \pm 930$  mg/cu m) with a mean weekly total concentration of  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) (Table 6). The total concentration usually diminished over a 60-min interval and averaged about a 35% loss of original concentration.

For the 15- and 60-min exposures to DF 2 smoke/exhaust, the average monthly exposure chamber temperatures during the 17 weeks ranged from  $67.9 \pm 3.9$  °F to  $78.1 \pm 4.3$  °F before smoke/exhaust entry,  $70.1 \pm 2.6$  °F to  $78.4 \pm 4.2$  °F 5 min after smoke/exhaust entry,  $70.2 \pm 6.1$  °F to  $77.8 \pm 4.4$  °F by 60 min after smoke/ exhaust entry.

The average monthly relative humidities in the chamber for the same periods ranged from  $47.1 \pm 2.4\%$ , to  $91.4 \pm 3.4\%$ ,  $47.1 \pm 2.4\%$  to  $91.5 \pm 3.4\%$ , and  $47.3 \pm 2.5\%$  to  $87.8 \pm 3.7\%$ . The average monthly ambient temperatures, exposure chamber temperatures, ambient relative humidities, and exposure chamber relative humidities are shown in Tables C-1 through C-5.

Any rises in temperature and relative humidity during DF2 smoke/exhaust emission into the animal exposure chamber were due to the heat and moisture generated from the M60A1 tank engine. Temperature rises usually dissipated significantly by 5 min after smoke/exhaust entry, while increases in relative humidity often remained.

The clouds produced by M60A1 tank-generated DF2 exhaust were less dense than those of the DF2 smoke/exhaust mixtures, and the odor was less acrid. As was the case with the clouds of DF2 smoke/exhaust, the total hydrocarbon concentration of the DF2 exhaust often dissipated over a 60-min interval. The average loss in concentration was about 44% of the original value.

The average weekly concentrations of hydrocarbon for DF2 exhaust over the 17-week exposure period (Table 6) ranged from  $0.002 \pm 0.002$  mg/l ( $2.0 \pm 2.0$  mg/cu m) to  $0.030 \pm 0.036$  mg/l ( $30.0 \pm 36.0$  mg/cu m) with an average weekly concentration of  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Average monthly temperatures in the chamber for the 15- and 60-min DF2 exhaust exposures ranged from  $67.8 \pm 3.1$  °F to  $78.4 \pm 3.7$  °F before exhaust entry,  $69.7 \pm 2.3$  °F to  $79.4 \pm 3.1$  °F 5 min after exhaust entry, and  $71.1 \pm 5.2$  °F to  $80.2 \pm 5.1$  °F by 60-min after exhaust entry.

Average monthly relative humidities in the chamber for DF2 exhaust were  $46.6 \pm 3.4\%$  to  $91.9 \pm 5.5\%$  before emission,  $47.0 \pm 2.7\%$  to  $92.8 \pm 4.8\%$  5 min after emission, and  $47.4 \pm 2.6\%$  to  $90.4 \pm 4.4\%$  by 60 min after emission entry.

Table 6. Weekly Total Hydrocarbon Concentrations of MG01 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> During Animal Subchronic Toxicity Studies by the Airborne Route of Exposure

Exposure week	DF2 Smoke/Exhaust-15 Min					DF2 Exhaust-60 min				
	Average weekly concn (mg/l)	Total weekly concn (mg/l)	Cumulative weekly ct (mg/min/cu m)	Average weekly concn (mg/l)	Total weekly concn (mg/l)	Cumulative weekly ct (mg/min/cu m)	Average weekly concn (mg/l)	Total weekly concn (mg/l)	Cumulative weekly ct (mg/min/cu m)	Average weekly concn (mg/l)
1	2.58 ± 0.69	12.88	38,700	0.007 ± 0.003	0.033	105	2.34 ± 0.22	11.70	140,400	0.004 ± 0.001
2	2.49 ± 0.22	12.46	76,050	0.007 ± 0.006	0.036	210	1.89 ± 0.24	9.46	253,800	0.020 ± 0.002
3	3.07 ± 0.27	9.22	122,100	0.003 ± 0.001	0.010	255	2.30 ± 0.55	6.91	391,800	0.003 ± 0.001
4	2.39 ± 0.70	14.32	157,950	0.003 ± 0.001	0.018	300	1.98 ± 0.56	11.86	510,600	0.002 ± 0.001
5	2.93 ± 0.52	17.59	201,900	0.004 ± 0.001	0.022	360	2.17 ± 0.56	13.03	640,800	0.002 ± 0.002
6	3.11 ± 0.79	15.54	228,550	0.009 ± 0.004	0.043	495	2.58 ± 0.63	12.90	795,600	0.007 ± 0.004
7	2.62 ± 0.19	13.10	287,500	0.018 ± 0.008	0.073	765	2.01 ± 0.20	10.03	916,200	0.012 ± 0.004
8	2.94 ± 1.34	14.71	331,950	0.030 ± 0.036	0.149	1,215	2.66 ± 1.01	13.30	1,075,800	0.015 ± 0.018
9	2.26 ± 0.39	11.32	365,850	0.008 ± 0.007	0.039	1,335	2.15 ± 0.26	12.88	1,204,800	0.006 ± 0.007
10	2.05 ± 0.26	8.20	396,600	0.006 ± 0.008	0.030	1,425	1.55 ± 0.24	6.21	1,297,800	0.005 ± 0.007
11	2.69 ± 1.24	13.43	436,950	0.004 ± 0.003	0.020	1,485	2.48 ± 1.06	12.40	1,466,600	0.003 ± 0.001
12	2.61 ± 0.72	13.06	476,100	0.004 ± 0.004	0.022	1,545	2.15 ± 0.69	10.76	1,575,600	0.004 ± 0.002
13	2.11 ± 0.40	10.55	507,750	0.003 ± 0.002	0.014	1,590	1.45 ± 0.24	7.24	1,662,600	0.002 ± 0.001
14	2.34 ± 0.26	11.70	542,850	0.002 ± 0.000	0.010	1,620	1.81 ± 0.18	9.04	1,771,200	0.002 ± 0.000
15	2.29 ± 1.45	11.44	577,200	0.002 ± 0.002	0.012	1,650	1.72 ± 1.35	8.58	1,874,400	0.002 ± 0.001
16	3.24 ± 0.93	16.18	625,800	0.004 ± 0.003	0.022	1,710	2.75 ± 0.90	13.77	2,039,400	0.003 ± 0.002
17	1.92 ± 0.24	9.62	654,600	0.003 ± 0.003	0.015	1,755	1.84 ± 0.24	9.20	2,149,800	0.003 ± 0.003

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust over a 17-week exposure period =  $2.340 \pm 0.450$  mg/l

( $2.340 \pm 450$  mg/cu m)

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust over a 17-week exposure period =  $0.006$  mg/l  $\pm$   $0.006$  mg/l

( $0.0 \pm 6.0$  mg/cu m).

Even though no animal was exposed to either DF2 smoke and/or exhaust longer than 13 weeks (65 days), the average total hydrocarbon concentration for either emission was based on a 17-week exposure period. This occurred because various animal groups were phased in weeks after the initiation of exposures, requiring a longer period of chamber exposures.

The average monthly ambient temperatures, exposure chamber temperatures, ambient relative humidities, and exposure chamber relative humidities are shown in Tables C-1 to C-5.

As was the case with the DF2 smoke/exhaust, both the chamber temperature and relative humidity rose during introduction of the DF2 exhaust clouds. This rise was due to the heat and condensation from the M60A1 tank engine. This rise in chamber temperature usually dissipated significantly by 5 min after the exhaust was introduced, but the relative humidities often remained elevated.

### 3.1.2 Gas Concentrations in the Chamber During Exposures.

Concentrations of carbon dioxide, nitrogen dioxide, sulfur dioxide, ethylene oxide, carbon monoxide, and oxygen were measured during animal exposures to DF2 smoke and/or exhaust. Regardless of the conditions of exposure, carbon dioxide never exceeded 1000 ppm, nitrogen dioxide  $4.4 \pm 3.9$  ppm, sulfur dioxide  $7.5 \pm 6.1$  ppm, ethylene oxide 25 ppm, or carbon monoxide  $16.1 \pm 6.3$  ppm. Oxygen concentrations never went below 20.0%. Actual values for the gas concentrations can be seen in Tables 7, 8, and 9.

Table 7. Gas Concentrations During Subchronic Toxicity Studies with M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> Disseminated by the Airborne Route

Exposure chamber gas	DF2 Smoke/Exhaust	DF2 Exhaust
	(Mean concentration with standard error)	(Mean concentration with standard error)
	ppm	
Carbon dioxide	(N* = 585) 1,000	(N = 585) 1,000
Nitrogen dioxide	(N = 17) $3.4 \pm 2.8$	(N = 17) $4.4 \pm 3.9$
Sulfur dioxide	(N = 16) $7.5 \pm 6.1$	(N = 17) $6.9 \pm 5.6$
Ethylene oxide	(N = 5) 25.0 <sup>c</sup>	(N = 14) 25.0 <sup>c</sup>

\*N = Number of samples tested.

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m).

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

<sup>c</sup>Ethylene oxide concentration was estimated as less than 25 ppm, since the lowest gradation of the Drager tube used was 25 ppm.

Table 8. Carbon Monoxide Concentrations During Subchronic Toxicity Studies With M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

Month	DF2 Exhaust 15 min	DF2 Exhaust 60 min	DF2 Smoke/Exhaust 15 min	DF2 Smoke/Exhaust 60 min
ppm				
August	7.0* $\pm$ 2.3	10.1 $\pm$ 3.8	8.9 $\pm$ 2.4	10.0 $\pm$ 2.5
September	9.7 $\pm$ 3.4	11.0 $\pm$ 4.8	9.4 $\pm$ 3.4	11.4 $\pm$ 3.8
October	15.2 $\pm$ 9.1	12.8 $\pm$ 3.7	13.0 $\pm$ 5.7	13.1 $\pm$ 4.3
November	16.1 $\pm$ 6.3	15.2 $\pm$ 6.4	14.8 $\pm$ 7.1	14.8 $\pm$ 5.5
December	14.7 $\pm$ 5.3	12.6 $\pm$ 4.1	12.2 $\pm$ 2.3	13.0 $\pm$ 5.6

\*Mean and Standard Deviation.

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust = 2.340  $\pm$  0.450 mg/l (2,340  $\pm$  450 mg/cu m).

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust = 0.006  $\pm$  0.006 mg/l (6.0  $\pm$  6.0 mg/cu m).

Table 9. Oxygen Concentrations<sup>a</sup> During Subchronic Toxicity Studies with M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>b</sup> and/or Exhaust<sup>c</sup>

Conditions	Aug	Sep	Oct	Nov	Dec
<u>Air Controls</u>					
60 minutes*					
Sample A	20.89 +0.01	20.87 +0.04	20.77 +0.52	20.89 +0.02	20.89 +0.04
Sample B	20.89 +0.01	20.87 +0.04	20.76 +0.52	20.90 +0.03	20.90 +0.02
<u>DF2 Exhaust-15 minutes</u>					
0 minutes*					
Sample A	20.92 +0.05	20.88 +0.05	20.77 +0.53	20.85 +0.06	20.89 +0.01
Sample B	20.92 +0.05	20.88 +0.05	20.77 +0.53	20.87 +0.09	20.89 +0.01
10 minutes*					
Sample A	20.88 +0.10	20.83 +0.08	20.60 +0.52	20.76 +0.14	20.79 +0.10
Sample B	20.86 +0.07	20.84 +0.08	20.63 +0.52	20.74 +0.10	20.86 +0.07
15 minutes*					
Sample A	20.89 +0.12	20.82 +0.07	20.57 +0.52	20.72 +0.12	20.85 +0.06
Sample B	20.89 +0.11	20.83 +0.08	20.55 +0.52	20.72 +0.10	20.82 +0.09

Table 9. (Continued)

Conditions	Aug	Sep	Oct	Nov	Dec
<u>DF2 Exhaust-60 Minutes</u>					
0 minutes*					
Sample A	20.88 <u>+0.06</u>	20.90 <u>+0.07</u>	20.78 <u>+0.52</u>	20.89 <u>+0.02</u>	20.89 <u>+0.02</u>
Sample B	20.86 <u>+0.08</u>	20.90 <u>+0.07</u>	20.78 <u>+0.52</u>	20.90 <u>+0.02</u>	20.89 <u>+0.02</u>
10 minutes*					
Sample A	20.78 <u>+0.12</u>	20.86 <u>+0.10</u>	20.63 <u>+0.52</u>	20.82 <u>+0.09</u>	20.83 <u>+0.08</u>
Sample B	20.77 <u>+0.14</u>	20.85 <u>+0.10</u>	20.62 <u>+0.54</u>	20.82 <u>+0.09</u>	20.80 <u>+0.15</u>
60 minutes*					
Sample A	20.88 <u>+0.05</u>	20.82 <u>+0.11</u>	20.59 <u>+0.51</u>	20.72 <u>+0.10</u>	20.79 <u>+0.09</u>
Sample B	20.90 <u>+0.05</u>	20.82 <u>+0.10</u>	20.58 <u>+0.53</u>	20.75 <u>+0.11</u>	20.79 <u>+0.13</u>
<u>DF2 Smoke/Exhaust-15 Minutes</u>					
0 Minutes*					
Sample A	20.91 <u>+0.11</u>	20.89 <u>+0.02</u>	20.90 <u>+0.04</u>	20.89 <u>+0.05</u>	20.87 <u>+0.08</u>
Sample B	20.90 <u>+0.13</u>	20.89 <u>+0.02</u>	20.90 <u>+0.04</u>	20.89 <u>+0.01</u>	20.87 <u>+0.08</u>
10 minutes*					
Sample A	20.82 <u>+0.10</u>	20.80 <u>+0.12</u>	20.79 <u>+0.11</u>	20.83 <u>+0.09</u>	20.78 <u>+0.08</u>
Sample B	20.82 <u>+0.10</u>	20.79 <u>+0.12</u>	20.80 <u>+0.12</u>	20.84 <u>+0.08</u>	20.81 <u>+0.10</u>
15 Minutes*					
Sample A	20.81 <u>+0.11</u>	20.78 <u>+0.12</u>	20.77 <u>+0.12</u>	20.80 <u>+0.10</u>	20.82 <u>+0.11</u>
Sample B	20.81 <u>+0.11</u>	20.77 <u>+0.13</u>	20.74 <u>+0.14</u>	20.81 <u>+0.09</u>	20.81 <u>+0.11</u>



Table 9. (Continued)

Conditions	Aug	Sep	Oct	Nov	Dec
DF2 Smoke/Exhaust-					
60 minutes					
0 minutes*					
Sample A	20.92 +0.04	20.89 +0.05	20.89 +0.04	20.91 +0.10	20.89 +0.03
Sample B	20.92 +0.03	20.88 +0.06	20.89 +0.04	20.89 +0.07	20.89 +0.02
10 minutes*					
Sample A	20.82 +0.11	20.83 +0.12	20.78 +0.10	20.80 +0.07	20.80 +0.13
Sample B	20.83 +0.11	20.81 +0.11	20.77 +0.11	20.80 +0.07	20.80 +0.15
60 Minutes*					
Sample A	20.82 +0.10	20.77 +0.11	20.69 +0.18	20.79 +0.11	20.78 +0.14
Sample B	20.83 +0.10	20.77 +0.13	20.70 +0.15	20.77 +0.11	20.80 +0.14

\*Indicates time of sampling during exposure.

<sup>a</sup>Values represent the mean with standard deviation of daily measurements made throughout each exposure month.

<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m).

<sup>c</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

### 3.1.3 Measurements of Particle Size (Geometric Mean Diameter).

At an average airborne concentration of 1.7 mg/l (1,700 mg/cu m), which was the average smoke/exhaust concentration obtained during the recording of these particle size measurements, the geometric mean diameter of the carbonaceous particles collected was 0.292  $\mu$ m with a geometric standard deviation of 5.252  $\mu$ m, Figure D-1.

At the average total hydrocarbon concentration (e.g., 0.006 + 0.006 mg/l or 6.0 + 6.0 mg/cu m) of DF2 exhaust generated during these exposures, the measurement of the geometric mean diameter of the carbonaceous particles was outside the sizing capabilities of the Sierra Model 2210K Cascade Impactor. The lowest definitive quantitation obtainable during sample collection was 0.1  $\mu$ m, at the flow rates used. Since no carbonaceous mass was detectable at the 0.1- $\mu$ m stage, it is reasonable to assume that the geometric mean diameter of the particles was less than 0.1  $\mu$ m.

### 3.2 Biological Effects of Exposures.

#### 3.2.1 Toxic Signs and Mortality.

No toxic signs were observed in B6C3F1 mice or Fischer 344 rats exposed to M60A1 tank-generated DF2 smoke and/or exhaust except for hypoactivity after exposure to the DF2 smoke/exhaust emissions for 60-min periods. This sign did not appear until after the 32nd daily exposure. It was observed after each additional exposure and was absent 24 hours later.

In addition, none of the spontaneous deaths that occurred among either species during the exposure or post-exposure periods could be attributed to DF2 smoke and/or exhaust exposures.

#### 3.2.2 Growth Rates of Mice and Rats.

The weekly group body weights of B6C3F1 mice and Fischer 344 rats obtained during and after the 13-week exposure to DF2 smoke and/or exhaust were compared per sex by the one-way ANOVA. Statistical significance was determined at the  $p \leq 0.05$  level. In addition, significant differences for any one week were further analyzed by Tukey's HSD procedure<sup>8</sup> to determine in which specific exposure group the significance existed.

Mean weekly body weights of animals from both species and each exposure condition were also plotted per sex and analyzed by the Analysis of Linear Regression (Least Squares Method). Slope differences between DF2 smoke/exhaust, DF2 exhaust, and control groups were then compared by calculation of Student's "t" values, with significance determined at the  $p \leq 0.05$  level.

When compared to control values, the only significant differences found in growth rate were in female mice exposed to DF2 exhaust for 15-min durations and 10-week-old male rats exposed to the same emission for the same time. The mice showed a lesser growth rate than the other groups (Figure E-2), while the rats showed a greater growth rate (Figure E-5). These differences were discounted, however, since animals exposed for 60 min to the same emission did not show significant differences from controls or any other exposed group.

throughout the study. The results of the Analysis of Linear Regression (Least Squares Method) are shown in Figures E-1 to E-6.

### 3.2.3 Hematological and/or Blood Chemical Values of B6C3F1 Mice and Fischer 344 Rats.

#### 3.2.3.1 Hematological Values.\*

##### a. B6C3F1 Mice.

The only hematological effects seen were in male mice exposed daily for 15 min to DF2 exhaust for 32 days (6 weeks). These included decreased levels of red blood cells, white blood cells, hematocrits, and hemoglobin (Table F-2).

No hematological effects were seen in male mice after 65 daily exposures to DF2 exhaust, or 30 days later. This held true regardless of the length of the daily exposure (e.g., 15 or 60 min).

Female mice manifested no hematological effects from exposure to DF2 exhaust, regardless of duration of daily or weekly exposure. This was also the case with both male and female mice exposed to DF2 smoke/exhaust.

##### b. Fischer 344 Rats.

Male rats exposed to DF2 exhaust showed decreases in lymphocytes after 32 daily 15- and 60-min exposures and also at 30 days after the 65th (60-min) exposure (Table F-3). Female rats manifested low neutrophils and increases in lymphocytes after 32 daily 60-min exposures to DF2 exhaust (Tables F-4 and F-5).

These effects were not seen in females after 65 daily 15- or 60-min exposures to DF2 exhaust, or at 30 days after the 65th exposure (Table F-4 and F-5).

Rats of either sex that were exposed for 15 or 60 min to DF2 smoke/exhaust for the periods tested showed no hematological changes.

#### 3.2.3.2 Blood Chemical Values.\*\*

##### a. Fischer 344 Rats.

Male rats that were exposed to DF2 exhaust for as many as 65 daily 15- or 60-min exposures showed no significant changes in blood chemistry except for increased albumin and glycogen levels (Table F-6) from the 60-min exposures.

\*The hematological data are shown in Tables F-2 to F-5. Complete nomenclature, in addition to the units of measurement, are found in Table F-1.

\*\*The blood chemical data from these studies are shown in Tables F-6 to F-13, Appendix F. Since the headings for each parameter measured are symbolized on these tables, the complete nomenclature, in addition to the units of measurement are found on Table F-1, Appendix F.

This effect occurred 30 days after the 65th (60-min) exposure, however, and was discounted due to the large variance in data. Female rats showed no significant changes in blood chemistry after 15-min exposures to the exhaust regardless of the length of the exposure.

Those animals, however, exposed daily for 60 min to DF2 exhaust for 32 days showed decreased creatinine and uric acid levels (Tables F-7 to F-8). Decreased uric acid, calcium, and phosphate levels (Tables F-8 and F-9), as well as an increase in albumin (Table F-8), were also seen in females exposed daily for 60 min over a 13-week period. These effects, however, were not evident by 30 days postexposure, and no other changes were observed in female rats exposed to DF2 exhaust.

Daily 60-min exposure of male rats for 32 days to DF2 smoke/exhaust caused a slight increase in sodium and a decrease in triglycerides (Tables F-10 to F-11). An increase in glucose was also evident after 65 daily 60-min exposures to DF2 smoke/exhaust (Table F-10).

In female rats, decreases in total protein and increases in albumin levels were found after 65 daily 15-min exposures to DF2 smoke/exhaust (Table F-12), and increases in glutamic oxalacetic transaminase levels (Table F-13) and glycogen (Table F-4) by 30 days after 65 daily 15-min exposures to the smoke/exhaust emission. No blood chemical changes were observed in female rats as a result of 32 or 65 daily exposures to DF2 smoke/exhaust.

#### 3.2.3.3 Significance of Hematological and Blood Chemical Findings.

The results of the hematological and blood chemical evaluations of B6C3F1 mice and Fischer 344 rats exposed to M60A1 tank-generated DF2 smoke and/or exhaust for 15- or 60-min daily exposures, up to 65 times, indicate that there were little, if any, hematological effects. There were, however, some changes in blood chemistry in rats of both sexes exposed to both types of emission. The trends for these changes, however, were not consistent with the sex of the animal or the type and duration of exposure. On this basis, any significance attributable to the DF2 smoke and/or exhaust exposures is doubtful.

#### 3.2.4 Changes in Blood Gases.

Blood levels carboxyhemoglobin found in Fischer 344 rats exposed to chamber concentrations of 1,720 mg/cu m of DF2 smoke/exhaust and 5.20 mg/cu m of DF2 exhaust for periods of 60 min did not exceed 11%. Control animals showed levels of 2%. Levels of carbon monoxide in the chamber during both emissions were 8 to 9 ppm.

These findings are consonant with those of Patty<sup>9</sup> who has shown that carboxyhemoglobin levels and carbon monoxide concentrations of these magnitudes are toxicologically safe. See Table 10 for results.

Table 10. Blood Levels of Carboxyhemoglobin Found in Fischer 344 Rats Exposed to M60A1 Tank-Generated DF2 Smoke and/or Exhaust Under Static Air Flow Conditions

Species	Exposure condition	Concentration mg/l	Exposure time min	Chamber concentration of carbon monoxide ppm	Amount of carboxy-hemoglobin in blood %
Fischer 344 rats	DF2 smoke/exhaust	1.72 (1,720 mg/cu m)	60	8-9	Control = 0-2 Exposed = 7-11
Fischer 344 Rats	DF2 Exhaust	0.0052 (5.20 mg/cu m)	60	8-9	Control = 0-2 Exposed = 7-10

\*Analysis - Dithionite Reduction Method<sup>2</sup>

### 3.2.5 Physiological.

#### 3.2.5.1 Pulmonary Responses in Rats Exposed to DF2 Smoke and/or Exhaust.

Tables G-1 to G-4 show that there were no significant differences in pulmonary function found in Fischer 344 rats after 15- or 60-min exposures to average total hydrocarbon concentrations of  $2.34 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of DF2 smoke/exhaust or  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m) of the exhaust. These rats included animals exposed to either type emission for 32 days, 65 days, or for 65 days + 30 days of observation.

#### 3.2.5.2 Physiological Evaluation of Rats After Exposure to DF2 Smoke and/or Exhaust.

The mean data, together with the standard deviation and the significance of the F rates from the ANOVA are given in Tables H-1 to H-3. The greatest number of differences in all data could be categorized according to sex, not dose, and include differences in weight, rectal temperature, tidal volume, minute volume, volume responses to 6% CO<sub>2</sub>, "t" wave amplitude on the EKG, and the treadmill run. Some of the differences, particularly the respiratory parameters, are related to the large differences in body size between male and female animals. Other differences due to sex, such as rectal temperature, have been observed in previous studies.<sup>3</sup>

In the 32-day exposure (Table H-1) a dose-related difference, a one-thousandth of a second increase, appeared in the "P" wave duration. The difference was determined by "t" test to be only between controls and rats exposed for 60-min to DF2 exhaust. Since this effect did not occur in longer exposures (e.g., 65 days), it appeared to have no physiological significance and was not correlated with any other condition or pathological change.

Another difference was found in heart rates as derived from electrocardiographic recordings. This effect was also determined by the "t" test and occurred between females exposed to for 15 and 60 min to DF2 smoke/exhaust for 32 days (Table H-1), but not between these animals and the controls. This difference, however, was not repeated in longer exposures and appeared to have no physiological significance. In addition, it did not correlate with any other condition, including pathological changes.

With the exception of these two findings, no other significant differences were found in the physiological measurements of the three test groups.

### 3.2.6 Behavioral Effects in Rats After Airborne Exposure to DF2 Smoke and/or Exhaust.

Results of daily 15- or 60-min exposures of Fischer 344 rats to average concentrations of M60A1 tank-generated DF2 smoke/exhaust ( $2,340 \pm 450$  mg/cu m) and DF2 exhaust ( $6.0 \pm 6.0$  mg/cu m) for up to 65 times are shown in Tables I-1 to I-3. The means and standard errors shown for the behavioral parameters were measured in animals exposed for 32 days (Table I-1), 65 days (Table I-2), and those held for 30 days after the 65-day exposures (Table I-3) under each of the test conditions.

These data, in addition to data analyzed by ANOVA and not presented here, indicated no statistically significant differences in behavioral responsiveness between control and test animals for any of the time periods or exposure intervals tested.

### 3.2.7 Teratogenic, Mutagenic, and Reproductive Effects.

#### 3.2.7.1 Toxic and Mutagenic Effects in Drosophila Melanogaster After Oral Exposure to DF2 (Diesel Fuel) or Airborne Exposure to DF2 Smoke/Exhaust.

Toxicity data from the exposure of Drosophila melanogaster to DF2 (diesel fuel) (orally) or to M60A1 tank-generated DF2 smoke/exhaust (by inhalation) are shown in Tables J-1 and J-2. In the oral exposure, the 10% DF2 (diesel fuel) mixture was the only concentration that caused significant mortality when compared to control values (Table J-1). The airborne exposure of Drosophila melanogaster to the DF2 smoke/exhaust produced, on a percentage basis, four to five times greater mortality than that experienced by the control insects (Table J-2).

Table J-3 shows that concentrations of 10%, 1%, and 0.1% of DF2 (diesel fuel), in the food medium tested, caused no mutagenic effects after oral exposure of Drosophila melanogaster for 72 hours. Similar findings (Table J-4), were seen when the same organisms were exposed daily for 60 min for 4 or 5 days to M60A1 tank-generated DF2 (diesel fuel) smoke/exhaust (average total hydrocarbon concentrations of  $2,340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)).

3.2.7.2 Teratogenic, Mutagenic, and Reproductive Effects in Sprague-Dawley Rats Exposed by the Airborne Route to DF2 Smoke and/or Exhaust.

3.2.7.2.1 Fetal Toxicity and Teratogenicity.

Using Chi-square analysis, it was determined that significantly more dams in the group exposed for 60 min to DF2 smoke/exhaust had two or more resorptions than those in other groups. Analysis with the Student's "t" test, however, showed no significant differences in body weights among the groups.

During gross examinations, it was observed that three fetuses in one litter from the DF2 smoke/exhaust group had major malformations. One female fetus exhibited exencephaly and was small, weighing only 2.39 gm. Another fetus, a male, had clubbed feet and spina bifida, and weighed only 1.97 gm (the average weight for normal litter mates was 2.97 gm). All three fetuses were stained for skeletal examinations. The first had a distorted cranium and short body; the second a divided cranium, cervical vertebra, and upper thoracic vertebra; and the third, a female, exhibited spina bifida when examined grossly. When examined visceraally, this animal also had hemorrhage of the olfactory bulbs, myeloschisis with distortion of the spinal cord, bilateral hydronephrosis and hydroureter, and schistocelia with evagination of intestine and fat. A fourth fetus in this litter, had a diaphragmatic hernia, and a fifth had greatly distended lateral ventricles with no apparent cranial distortion. One fetus in the group exposed to DF2 exhaust had intestines extruding at the site of the umbilicus. No major abnormalities were seen in the control (tank engine noise) group.

There were a few visceral variations that were exhibited across groups; these included enlarged renal pelvises and abdominal hematomas. In the tank engine noise and DF2 exhaust groups, others were seen that involved the eyes and heart. No visceral variations were seen in the group exposed to DF2 smoke/exhaust.

Minor skeletal variations observed across groups were more numerous. They included sites of retarded ossification that were concentrated in the vertebral column, ribs, and sternum. The percentage of fetuses showing sites of low ossification were as follows:

<u>Controls (tank engine noise)</u>	<u>DF2 Exhaust</u>	<u>DF2 Smoke/exhaust</u>
65%	79%	81%

It should be noted that the tank engine noise had an effect in the above findings. This statement is based on data from both historical and "static" controls (e.g., six control animals that were retained under the same climatic and procedural conditions as the exposed animals) that showed only a 7% incidence of low skeletal ossification.\*

\*Personal communication. Mr. William C. Starke, Biosciences Branch, Toxicology Division, Research Directorate, Chemical Research and Development Center. 1984.

### 3.2.7.2.2 Dominant Lethal Mutation

With only two exceptions, all males successfully impregnated at least one of the available females in each of the two post-exposure mating periods. The male in the control group (tank noise) failed to impregnate either female in the 2nd week of post-exposure mating. The male in the 60-min DF2 exhaust group failed to impregnate either female in either of the two post-exposure mating periods. Pregnancies were ascertained on the estimated 18th day of gestation (assuming conception occurred in the first 24 hours of cohabitation). From the recorded data were calculated: the mating index, corpora lutea index, implantation index, preimplantation loss index, fetal index, resorption index, ratio of nonviable to viable fetuses, percentage of dams with one or more nonviable fetuses, and percentage of dams with two or more nonviable fetuses.

The number of dams with one or more resorptions was significant, > 55% in the 2nd week matings for the 15-min DF2 exhaust group. However, none of the other parameters showed any significance.

### 3.2.7.2.3 Reproduction in a Single Generation.

In this portion of the study, mating, the period of gestation, delivery, and care of neonates were of similar quality across groups. It was determined, however, that the average body weight of male pups at day 1 in the 60-min DF2 exhaust group was significantly lower than that of controls. In addition, even though the litters had been culled to a maximum of 10 pups each on day 4, average body weight on day 7 for female pups in the 60-min DF2 exhaust group was significantly lower than the average body weights of the control group. By day 21, no significant differences in the average body weights were apparent for any of the groups. Calculations of the viability and lactation indices also showed no significant differences between litters from the control and exposed groups.

Necropsies of 21-day-old pups from the control group showed one male and one female runt, four females with hydronephrosis, and one female with unilateral anophthalmia. Examination of the pups from the group exposed for 15 min to DF2 exhaust showed one male and one female runt, one female whose paracardial sac contained fluid, two females with hydronephrosis, and one male with an underdeveloped testicle. In the group exposed for 60 min to DF2 exhaust, one female had an unusually short body, and two males and four females had hydronephrosis.

In those animals exposed for 15 min to DF2 smoke/exhaust, one female manifested hydronephrosis. In other animals exposed for 60 min to DF2 smoke/exhaust, one female was a runt; two males and four females showed hydronephrosis, and one male had malformed eyelids.

The complete findings from studies designed to determine the teratogenic, mutagenic, and reproductive effects in Sprague Dawley rats exposed by inhalation to M60A1 tank-generated DF2 (diesel fuel) smoke and/or exhaust are described by William C. Starke et al.\*

\*Starke, W. C., Pelterin, R. J., and Burnett, D. C. Diesel Fuel-2, Teratogenicity, Mutagenicity and Effects on Reproduction in a Single Generation in Rats. In preparation.



3.2.8 Gross and/or Histopathological Findings from Mice and Rats Exposed to DF2 Smoke and/or Exhaust.

3.2.8.1 Gross Patholoical Effects.

B6C3F1 mice and Fischer 344 rats were exposed daily by aerosol as many as 65 times for 15 or 60 min to average total hydrocarbon concentrations of  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of DF2 smoke/exhaust or  $0.006 \pm 0.006$  mg/T ( $6.0 \pm 6.0$  mg/cu m) of exhaust and showed no significant gross pathological lesions at necropsy. This was also true for those animals exposed 65 times at each exposure condition and held 30 days post-exposure. Histopathological findings were also insignificant and are described verbatim from the evaluatory remarks of the examining histopathologist.

3.2.8.2 Histopathological Effects.

Species: B6C3FI Mice

Sex: Male

Condition: 32-day exposure, sacrifice following the last exposure

(Tables B-1 to B-2)

The incidence of mild to moderate pulmonary congestion was increased in animals exposed to DF2 exhaust for 15- and 60-min durations and those exposed for 15-min durations to DF2 smoke/exhaust. These lesions represented a slight and irregular response from exposure to DF2 exhaust or the smoke/exhaust combination.

Condition: 65-day exposure, sacrifice following the last exposure

(Tables B-3 to B-4)

The incidence of pulmonary congestion was increased in animals exposed for 15- or 60-min durations to either DF2 smoke and/or exhaust. The lesions represented a low intensity response from exposure to both DF2 smoke and/or exhaust emissions.

Condition: 65-day exposure, sacrifice 30 days after the last exposure

(Tables B-5 to B-6)

There were no lesions that could be associated with either the DF2 smoke and/or exhaust exposures.

Species: B6C3F1 Mice

Sex: Females

Condition: 32-day exposure, sacrifice following the last exposure

(Tables B-7 to B-8)

The incidence of pulmonary congestion was increased in animals exposed to DF2 smoke/exhaust for 15- and 60-min periods. This increase represented a slight response from exposure to DF2 smoke/exhaust.

Condition: 65-day exposure, sacrifice following the last exposure

(Tables B-9 to B-10)

The incidence of pulmonary congestion was increased in animals exposed to DF2 exhaust for 15- and 60-min periods and those exposed to DF2 smoke/exhaust for 15-min durations. Mild to moderate turbinate congestion was also found in one animal exposed to DF2 exhaust for 15-min durations, one exposed to the same emission for 60-min periods, and in three rats exposed to DF2 smoke/exhaust for 15-min periods. These pulmonary and turbinate lesions represented a low-grade, irregular response from exposure to DF2 smoke and/or exhaust.

Condition: 65-day exposure, sacrifice 30 days after the last exposure

(Tables B-11 to B-12)

No lesions could be associated with the DF2 smoke and/or exhaust exposures.

Summary and Conclusions. Gross and/or Histopathological Evaluation of B6C3F1 Mice After Exposure to DF2 Smoke and/or Exhaust Emissions.

Vascular congestion in turbinates and lungs was found more frequently in males and females exposed to DF2 smoke/exhaust. These lesions occurred sporadically, were of low intensity, and were not related to the duration of exposure; consequently, their biological significance is questionable. No exposure-related lesions were found in mice of either sex following a 30-day post-exposure recovery.

Species: Fischer 344 Rats

Sex: Males

Condition: 32-day exposure, sacrifice following the last exposure

(Tables B-13 to B-14)

Mild to moderate chronic tracheitis was observed in two males exposed to DF2 smoke/exhaust during the 15-min exposures and in one animal exposed to DF2 exhaust during the 60-min exposures. No tracheal lesions, however, were found in males as a result of the 60-min exposures to DF2 smoke/exhaust.

Minimal to mild chronic active peribronchiolitis was seen in one animal exposed to DF2 exhaust and in one exposed to DF2 smoke/exhaust during the 15-min exposures. Another animal exposed to DF2 exhaust showed the same effect from the 60-min exposures.

Minimal to moderate hemorrhage was observed in the turbinates of one animal in each type emission during the 15-min exposures. Two animals exposed to DF2 exhaust and one exposed to DF2 smoke/exhaust also manifested turbinate hemorrhage from the 60-min exposures.

Conditions: 65-day exposure, sacrifice following the last exposure

(Table B-15 to B-16)

Mild to moderate hemorrhage was seen in the turbinates of two animals exposed to DF2 smoke/exhaust and in one animal exposed to DF2 exhaust during the 15-min exposures. One animal in each type of emission also showed turbinate hemorrhage during the 60-min exposures.

Condition: 65-day exposure, sacrifice 30-day after the last exposure

(Table I-17 to I-18)

During the 15-min exposures, mild to moderate hemorrhage was seen in the turbinates of two animals exposed to DF2 smoke/exhaust. The same effect was also seen in one animal exposed to DF2 exhaust during the 60-min exposures.

Species: Fischer 344 Rats

Sex: Females

Condition: 32-day exposure, sacrifice following the last exposure

(Tables B-19 to B-20)

Minimal to mild chronic active peribronchiolitis was observed in one animal each during the 15- and 60-min exposures to DF2 smoke and/or exhaust. Moderate to mild hemorrhage in the turbinates was also noted in one animal each exposed to DF2 smoke and/or exhaust during the 15-min exposures and in one animal during the 60-min exposures to DF2 exhaust.

Condition: 65-day exposure, sacrifice following the last exposure

(Tables B-21 to B-22)

Minimal to mild peribronchiolitis was observed in one animal exposed to DF2 exhaust and one to DF2 smoke/exhaust during the 15-min exposures. One animal exposed to DF2 smoke/exhaust for 60-min durations also had peribronchiolitis.

Condition: 65-day exposure, sacrifice 30 days after the last exposure

(Tables B-23 to B-24)

Minimal chronic peribronchiolitis was observed in one animal from 60-min exposures to DF2 exhaust.

### Summary and Conclusions. Gross and/or Histopathological Evaluation of Fischer 344 Rats After Exposure to DF2 Smoke and/or Exhaust.

Inflammatory and vascular lesions in turbinates, trachea, and lung were observed more frequently in rats of both sexes exposed to DF2 smoke/exhaust. These lesions occurred sporadically, were generally of low intensity, and not related to the duration of exposure. Consequently, the biological significance of the changes is doubtful. It should be noted, however, that there were no exposure-related inflammatory lesions in any of these animals after the 30-day recovery period.

#### 3.2.9 Ratios of Organ to Total Body Weights of B6C3F1 Mice and Fischer 344 Rats After Airborne Exposure to DF2 Smoke and/or Exhaust.

B6C3F1 mice and Fischer 344 rats, exposed for 15 or 60 min by aerosol to  $2,340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of DF2 smoke/exhaust or  $0.006 \pm 0.006$  mg/T ( $6.0 \pm 6.0$  mg/cu m) of exhaust and held 30 days post-exposure, had no significant changes in the weight ratios of hearts, lungs, livers, kidneys, and gonads to total body weights. Results of these findings are shown in Tables K-1 to K-4.

#### 4. DISCUSSION

In this subchronic toxicity study, B6C3F1 mice and Fischer 344 rats were exposed by the airborne route to average total hydrocarbon concentrations of  $2,340 \pm 450$  mg/sq m of M60A1 tank-generated DF2 (diesel fuel) smoke/exhaust and  $6.0 \pm 6.0$  mg/cu m of DF2 exhaust for as many as 65 daily 15- or 60-min exposures. These exposures emulated the average field concentrations that personnel would experience while positioned approximately 6,000 meters downwind from a tank during an atmospheric inversion. The assumption was that the tank was standing still, with no load on the engine and its Vehicle Engine Exhaust Smoke System (VEESS) operating at maximal smoke-generating efficiency. These DF2 smoke and/or exhaust concentrations would also not be unusual for personnel to experience while standing closer to the tank (e.g., 10 meters distance from the emission source) on a windy day.

There were no consistent trends in weight loss or retarded growth in either animal species during the 13-week exposure or 30-day post-exposure period. No toxic signs were exhibited except hypoactivity in both mice and rats exposed to the daily 60-min DF2 smoke/exhaust emissions. This sign did not appear until after the 32nd daily exposure and disappeared within 24 hours after each exposure. Its occurrence could have been indicative of slight respiratory distress.

There were also no spontaneous deaths among either species that could be attributed to the DF2 smoke and/or exhaust exposures.

No consistent hematological or blood chemical effects were observed as a result of the daily 15- or 60-min exposures to DF2 (diesel fuel) smoke and/or exhaust. Blood gas levels (e.g., carboxyhemoglobin) of rats exposed for 60-min durations to DF2 smoke and/or exhaust did not exceed 11%. This is equivalent to the carboxyhemoglobin levels found in heavy cigarette smokers.<sup>10</sup>

Pulmonary function tests in rats showed no significant effects from exposures to DF2 smoke and/or exhaust, indicating normal estimated pulmonary compliance and/or resistance response. Measurements of rectal temperature, electrocardiographic (EKG) responses, blood pressure, pulmonary ventilation (e.g., response to CO<sub>2</sub> stimulation), physical performance, and reflex activity were generally within acceptable limits.

Normal responses to CO<sub>2</sub> stimulation indicated that the chemoreceptors of the aortic and carotid bodies and the respiratory centers were functioning normally. In addition, lack of significant changes in blood pressure, EKG, or heart rate of the rats showed no cardiotoxic effects, which was borne out by pathological examination (e.g., insignificant cardiovascular lesions).

There were no abnormal behavioral effects in rats from the exposures to DF2 smoke and/or exhaust as evidenced by the results of the Spontaneous Activity Test (SAT) and Passive Avoidance Test (PAT).

There were no gross pathological lesions of any significance in mice and rats exposed to either type of DF2 (diesel fuel) emission for the designated exposure conditions. Ratios of organ-to-body weights were also normal for the hearts, lungs, livers, kidneys, and gonads of mice and rats exposed for 65 days to the DF2 smoke and/or exhaust emissions and held 30 days post-exposure.

Histopathological evaluation of mice and rats exposed to DF2 smoke and/or exhaust emissions showed inflammatory vascular congestion in the turbinates and lungs of both species and in the trachea of rats. These effects were observed after 32 and 65 (15- or 60-min) daily exposures to either emission, but were absent by 30 days after the 65th exposure. However, these lesions were of low intensity, occurred sporadically, and were usually not dose related. This fact makes the biological significance of these changes very questionable.

Although the oral ingestion by Drosophila melanogaster of 10% concentrations of DF2 diesel fuel in ethanol caused significant mortality, the mixture was not mutagenic. Airborne exposure of the same species to average total hydrocarbon concentrations of  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of DF2 smoke/exhaust for 4 to 5 daily 60-min exposures also produced significant mortality. This mode of exposure, however, was also found to be nonmutagenic.

The dominant lethal mutation screen and a single-generation reproductive test with Sprague-Dawley rats, exposed by the airborne route to M60A1 tank-generated DF2 smoke and/or exhaust, demonstrated negative effects. Tests for fetal toxicity and teratogenesis, however, in pregnant Sprague-Dawley dams exposed daily for 60 min to DF2 smoke and/or exhaust proved positive. Sites of retarded ossification were found to be significantly higher in the vertebral columns, ribs, and sternums of pups exposed to either emissions than in those animals exposed to tank noise only. Apparently the stress of the exposure conditions caused significant weight loss in the dams, resulting in these fetal anomalies.

The gases generated in the chamber during these exposures should have been within safe limits. Regardless of whether DF2 smoke/exhaust or exhaust only was used, carbon dioxide levels never exceeded 1000 ppm, carbon monoxide  $16.1 \pm 6.3$  ppm, nitrogen dioxide  $4.4 \pm 3.9$  ppm, sulfur dioxide  $7.5 \pm 6.1$  ppm, and ethylene oxide 25 ppm, and oxygen concentrations never went below 20.0%.

According to Patty<sup>9</sup> the concentrations of carbon dioxide and monoxide should have been safe for the exposure durations maintained. In addition, the carboxyhemoglobin blood levels in rats exposed to DF2 smoke and/or exhaust verify that the carbon monoxide concentrations were safe.

The concentrations of nitrogen dioxide, sulfur dioxide, and ethylene oxide in the exposure chambers should also have been safe. The Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal With Toxicity and Hazard Data<sup>11</sup> reports 5 ppm as the threshold limit value (TLV) for nitrogen dioxide. It also reports the inhalation LcLo (lowest published lethal concentration) for mice to be 250 ppm/30 min and the LC50 for rats to be 67 ppm/4 hr. In addition, Weedon et al.<sup>12</sup> exposed mice and guinea pigs to concentrations of 10, 25, 33, 65, 100, 150, 300, and 1000 ppm of sulfur dioxide and found that the gas caused no significant effects in either species at concentrations of 33 ppm or less. They also determined the median lethal concentrations for sulfur dioxide in mice: 130 ppm for 24 hr, 340 ppm for 6 hr, 610 ppm for 1 hr, and 1350 ppm for 10 min. Finally, Verschueren in his handbook of Environmental Data on Organic Chemicals<sup>13</sup> reports the inhalation LC50 for ethylene oxide in mice to be 835 ppm/4 hrs and in rats, 1,460 ppm/4 hr. The concentrations of ethylene oxide measured in these studies never reached such magnitude.

Normal atmospheric oxygen is 20.9%. During these DF2 smoke and/or exhaust studies, the mean oxygen concentrations were never lower than 20.0% which is sufficient to prevent oxygen deficiency.

One other facet of this study that should be addressed is the particulate sizes of both emissions. Schreck<sup>14</sup> reported particle size as one reason why increased exposure to particulates derived from diesel engine exhaust may constitute a potential health hazard. His work showed that the mass median diameter of carbonaceous particles is primarily in a size range of 0.2 to 0.3  $\mu\text{m}$ , which could allow for deposition and possible retention in the deep compartments of the lung.

The geometric mean diameters of particles from the DF2 smoke/exhaust emissions measured 0.292  $\mu\text{m}$  (Figure K-1). The particle size of the DF2 exhaust emissions was immeasurable, indicating that the preponderance was less than 0.1  $\mu\text{m}$ , which was the most definitive quantitation obtainable in these studies at the sampling flow rates used. Based on Schreck's hypothesis, therefore, the particles developed from either type emission generated in our studies would be prime candidates for deposition and retention in the lower compartments of the lung.

We addressed the toxic effects of repeated airborne exposures, under static airflow conditions, to average total hydrocarbon concentrations of  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of DF2 (diesel fuel) smoke/exhaust and  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m) of DF2 exhaust. With the exception of hypoactivity found in mice and rats exposed to the 60-min DF2 smoke/exhaust emission and the skeletal anomalies found in rat pups exposed to DF2 smoke and/or exhaust 60-min, all other parameters measured proved essentially negative.

In summary, the tests encompassed an approximate 3-month daily exposure period. However, even with the longevity of the exposures and the apparent dearth of adverse effects in the animals, it is recommended that personnel exposed to even these concentrations of VEES-generated DF2 (diesel fuel) smoke and/or exhaust emissions be masked regardless of the duration or incidence of exposure.

## 5. CONCLUSIONS

- B6C3F1 mice and Fischer 344 rats were exposed by the airborne route under static airflow conditions to average total hydrocarbon concentrations of  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m) of M60A1 tank-generated DF2 (diesel fuel) smoke/exhaust or  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m) of exhaust only. Daily exposures lasted 15 or 60 min and lasted for periods up to 13 weeks.

- Two significant toxicological effects were found: (a) the manifestation of hypoactivity, a toxic sign that was observed in both species after daily 60-min exposures to the DF2 smoke/exhaust mixture, and (b) skeletal anomalies in rat pups delivered from pregnant dams that had been exposed daily for 60 min to DF2 smoke and/or exhaust emissions during the 6th to 15th day of gestation.

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APPENDIX A

CHEMICAL AND PHYSICAL QUALITY OF DF2 DIESEL FUEL USED IN  
SUBCHRONIC INHALATION TOXICITY STUDIES

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Table A-1. Property Requirements for DF2 Diesel Fuel Used in High-Speed Engines Per Federal Specification VV-F800B

Properties	Diesel Fuel Grade-DF2 Values CONUS <sup>1/</sup>	Values OCONUS <sup>1/</sup>
Gravity, °API	Report <sup>2/</sup>	32.9 to 41.0
Flash Point, °F (°C) min	125 (51.7)	133 (56)
Cloud Point, °F (°C) min max <sup>3/</sup>	Report <sup>2/</sup>	<sup>4/</sup>
Pour Point, °F (°C) max <sup>5/</sup>	Report <sup>2/</sup>	<sup>6/</sup>
Kinematic Viscosity @ 100 °F (37.8 °C), cSt	2.0 to 4.3	1.8 to 9.5
Distillation, °F (°C):		
50% evaporated	Report <sup>2/</sup>	Report <sup>2/</sup>
90% evaporated, max	640 (338)	675 (337)
End point, max	700 (371)	700 (371)
Carbon residue on 10% bottoms, % wt, max <sup>7/</sup>	0.35	0.20
Sulfur, % wt, max	0.50	0.70
Copper strip corrosion, 3 hr @ 122 °F (50 °C), max rating	3	1
Ash, % wt, max	0.01	0.02
Water & sediment, % max	0.01	0.01
Accelerated stability, total insolubles mg/100 ml max <sup>8/</sup>	1.5	1.5
Neutralization number TAN, max	---	0.10
Particulate contamination mg/liter, max	8	8
Cetane number, min	45	45

<sup>1/</sup>CONUS stands for Continental U.S., OCONUS stands for outside Continental U.S.

<sup>2/</sup>Federal Specification, VV-F-800B. Commissioner, Federal Supply Services, General Services Administration, April 2, 1975. Copies may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC, 20402.

<sup>3/</sup>Cloud point - temperature at which solid substances begin to separate from the fuel under conditions of American Society for Testing and Material Method (ASTM) D-2500.

<sup>4/</sup>DF2 destined for Europe and S. Korea shall have a maximum limit of 9 °F (-13 °C). For other OCONUS areas, the maximum limit must be specified by the procuring activity.

<sup>5/</sup>Pour point - lowest temperature at which fuel will pour or flow under conditions of ASTM Method D97.

<sup>6/</sup>DF2 destined for Europe and S. Korea shall have a maximum limit of 0 °F (-18 °C). For other OCONUS areas, the maximum limit must be specified by the procuring activity.

<sup>7/</sup>The maximum limits do not apply for samples containing cetane improvers. In those instances, the test must be performed on the base fuel blend.

<sup>8/</sup>This requirement is applicable only for military bulk deliveries intended for tactical, OCONUS, or long-term storage (greater than 6 months) applications (i.e., Army depots, etc.).

Table A-2. Chemical and Physical Properties of the DF2 Diesel Fuel Used in Studies to Determine the the Subchronic Inhalation Toxicity of Smoke and/or Exhaust Generated by the M60A1 Tank

Drum no.	Sample no.	Water and sediment <sup>a</sup> %	Cloud point <sup>b</sup> °F	Kinematic viscosity <sup>c</sup> (centistokes)	Flash point <sup>d</sup> °F	Ash <sup>e</sup> %
1	1	0.005	+12	2.51	176	0.000
	2	0.003	+16	2.40	167	0.000
2	1	0.008	+18	2.59	185	0.000
	2	0.008	+16	2.59	185	0.000
3	1	0.003	+38	2.66	176	0.000
	2	0.003	+36	2.63	176	0.000
4	1	0.003	+38	2.64	158	0.000
	2	0.003	+36	2.57	167	0.000
5	1	0.003	+34	2.64	176	0.004
	2	0.003	+38	2.58	167	0.008
6	1	0.004	+40	2.86	176	0.000

<sup>a</sup>Water and sediment. None of the samples contained visible quantities of water and sediment. Karl Fischer water ranged from 0.000% to 0.008%. These values are within specified limits.

<sup>b</sup>Cloud point. All DF2 fuel samples met the requirements of Federal specification VV-F-800B.

<sup>c</sup>Kinematic viscosity. All DF2 samples met viscosity requirements.

<sup>d</sup>Flash point. Flash points of all samples met the requirements of Federal Specification VV-F-800B.

<sup>e</sup>Ash. Ash content of all DF2 samples fell within the requirements of Federal Specifications VV-F-800B.

Table A-3. Distillation Ranges of the DF2 Diesel Fuel Used in Studies to Determine the Subchronic Inhalation Toxicity of Smoke and/or Exhaust Generated by the M60A1 Tank

Drum no.	Sample no.	Initial boiling point	Distillation Range* (°F)			
			10% Evap.	50% Evap.	90% Evap.	End point
1	1	370	407	494	609	685
	2	364	407	491	610	684
2	1	360	410	516	624	695
	2	360	410	515	620	691
3	1	360	410	518	623	692
	2	360	411	515	618	694
4	1	365	415	519	622	693
	2	365	412	517	623	696
5	1	365	412	523	629	697
	2	365	413	517	630	696
6	1	380	429	520	619	697

\*All DF2 fuel samples met the requirements of Federal Specification VV-F-800B.

Blank



**APPENDIX B**

**HISTOPATHOLOGICAL EFFECTS OBSERVED IN B6C3F1 MICE AND FISCHER 344 RATS  
DURING AND AFTER AIRBORNE EXPOSURE TO M60A1 TANK-GENERATED DF2  
(DIESEL FUEL) SMOKE AND/OR EXHAUST**

Blank

Table B-1. Histopathological Effects in Male B6C3F1 Mice After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smokeb Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smokeb Exhaust (60 min)			
		0000	0001	0002	0003	0004	0005	0006	0007	0008	0009	0010	0011	0012	0013	0014	0015	0016	0017	0018	0019
Spinal Cord		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal																					
Peribronchiolitis, multifocal, chronic, active																					
Hemorrhage, multifocal																					
Congestion, diffuse																					
Congestion, multifocal																					
Kidney																					
Lymphocytosis, interstitial, multifocal																					
Nephropathy, unilateral																					
Liver																					
Gall Bladder																					
Spleen																					
Hydrothorax																					
Salivary Gland																					
Parathyroid Lymph Node																					
Thymic Lymph Node																					
Spleen																					
Heart																					
Myocarditis, multifocal, chronic																					

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Exposure: 32 days  
Post exposure: 0 days

aAverage total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l

bAverage total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 + 0.450 \text{ mg/l}$

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Table B-2. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smoke <sup>b</sup> Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smoke <sup>b</sup> Exhaust (60 min)			
		006	008	012	010	006	008	012	010	006	008	012	010	006	008	012	010	006	008	012	010
Thigh Muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulation tissue, focal																					
Sciatic Nerve	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, focal																					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large Intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematodiasis																					
Turbinates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, multifocal																					
Congestion, multifocal																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mammary Gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal Vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Exposure: 65 days

Post exposure: 0 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l

Table B-3. Histopathological Effects in Male B6C3F1 Mice After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

	Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smoke Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smoke Exhaust (60 min)			
			01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04
Spinal Cord			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pneumonia, focal, chronic, active			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Peribronchiolitis, multifocal, chronic, active			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hemorrhage, focal			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kidney			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell Bladder			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary Gland			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid Lymph Node			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Lymph Node			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Long Marrow			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thigh Muscle			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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Exposure: 65 days  
Post exposure: 30 days

$$\text{bAverage total hydrocarbon concentration of DF2 smoke/exhaust} = 2.340 + 0.450 \text{ mg/l}$$



Table B-4. Histopathological Effects in Female B6C3F1 Mice After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

	Animal No.	No. of exposure days	Air Controls (60 min)		DF2a Exhaust (15 min)		DF2 Smoke <sup>b</sup> Exhaust (15 min)		DF2a Exhaust (60 min)		DF2 Smoke <sup>b</sup> Exhaust (60 min)	
			+	-	+	-	+	-	+	-	+	-
Spinal Cord			0	0	0	0	0	0	0	0	0	0
Brain			0	0	0	0	0	0	0	0	0	0
Eye			0	0	0	0	0	0	0	0	0	0
Pituitary			0	0	0	0	0	0	0	0	0	0
Larynx			0	0	0	0	0	0	0	0	0	0
Trachea			0	0	0	0	0	0	0	0	0	0
Esophagus			0	0	0	0	0	0	0	0	0	0
Adrenal			0	0	0	0	0	0	0	0	0	0
Thyroid			0	0	0	0	0	0	0	0	0	0
Parathyroid			0	0	0	0	0	0	0	0	0	0
Lung			0	0	0	0	0	0	0	0	0	0
Lymphocytosis, peribronchiolar, multifocal			0	0	0	0	0	0	0	0	0	0
Peribronchiolitis, multifocal, chronic			0	0	0	0	0	0	0	0	0	0
Congestion, diffuse			0	0	0	0	0	0	0	0	0	0
Congestion, multifocal			0	0	0	0	0	0	0	0	0	0
Kidney			0	0	0	0	0	0	0	0	0	0
Lymphocytosis, interstitial, multifocal			0	0	0	0	0	0	0	0	0	0
Liver			0	0	0	0	0	0	0	0	0	0
Gall Bladder			0	0	0	0	0	0	0	0	0	0
Gleason			0	0	0	0	0	0	0	0	0	0
Thymus			0	0	0	0	0	0	0	0	0	0
Salivary Gland			0	0	0	0	0	0	0	0	0	0
Nodular lymph Node			0	0	0	0	0	0	0	0	0	0
Parathyroid lymph Node			0	0	0	0	0	0	0	0	0	0
Heart			0	0	0	0	0	0	0	0	0	0
Bladder			0	0	0	0	0	0	0	0	0	0
Prostate			0	0	0	0	0	0	0	0	0	0
Rectum			0	0	0	0	0	0	0	0	0	0
Colon			0	0	0	0	0	0	0	0	0	0
Stomach			0	0	0	0	0	0	0	0	0	0
Small Intestine			0	0	0	0	0	0	0	0	0	0
Large Intestine			0	0	0	0	0	0	0	0	0	0
Uterus			0	0	0	0	0	0	0	0	0	0
Vagina			0	0	0	0	0	0	0	0	0	0
Testis			0	0	0	0	0	0	0	0	0	0
Spleen			0	0	0	0	0	0	0	0	0	0
Adipose			0	0	0	0	0	0	0	0	0	0
Heart Muscle			0	0	0	0	0	0	0	0	0	0
Skeletal Muscle			0	0	0	0	0	0	0	0	0	0
Brain			0	0	0	0	0	0	0	0	0	0
Heart			0	0	0	0	0	0	0	0	0	0
Liver			0	0	0	0	0	0	0	0	0	0
Stomach			0	0	0	0	0	0	0	0	0	0
Small Intestine			0	0	0	0	0	0	0	0	0	0
Large Intestine			0	0	0	0	0	0	0	0	0	0
Uterus			0	0	0	0	0	0	0	0	0	0
Vagina			0	0	0	0	0	0	0	0	0	0
Testis			0	0	0	0	0	0	0	0	0	0
Spleen			0	0	0	0	0	0	0	0	0	0
Adipose			0	0	0	0	0	0	0	0	0	0
Heart Muscle			0	0	0	0	0	0	0	0	0	0
Skeletal Muscle			0	0	0	0	0	0	0	0	0	0

Exposure: 32 days  
Post exposure: 0 days

$$\text{Average total hydrocarbon concentration of DF2 exhaust} = 0.006 + 0.006 \text{ mg/l}$$
$$^b \text{Average total hydrocarbon concentration of DF2 smoke/exhaust} = 2.340 + 0.450 \text{ mg/l}$$

\*Denotes spontaneous death. Cause of death not related to exposure.

**\*\*Male accessory organs present - tissue processing error.**

Table B-5. Histopathological Effects in Female B6C3F1 Mice After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smoke Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smoke Exhaust (60 min)			
		00000	00001	00002	00003	00004	00005	00006	00007	00008	00009	00010	00011	00012	00013	00014	00015	00016	00017	00018	00019
Spinal Cord		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal		1	2	2	2	1	1	2	2	2	2	2	2	2	1			1	2	2	2
Peribronchiolitis, multifocal, chronic, active								2											2		
Congestion, diffuse																					
Congestion, multifocal		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Kidney		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, interstitial, multifocal																					
Liver		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gall Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sialadenitis, focal, chronic																					
Mucibular Lymph Node		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Lymph Node		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone Marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B-5. (Continued)

[illegible]

**Exposure: 65 days**

Post exposure: 0 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l

Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l

Table B-6. Histopathological Effects in Female B6C3F1 Mice After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

	Animal No.	No. of exposure days	Air Controls (60 min)			DF2a Exhaust (15 min)			DF2 Smokeb Exhaust (15 min)			DF2a Exhaust (60 min)			DF2 Smokeb Exhaust (60 min)		
			012345	012345	012345	012345	012345	012345	012345	012345	012345	012345	012345	012345	012345	012345	012345
Spinal Cord			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal			11	111	111	11	111	1	11	11	11	11211	11	111	11	11	11
Bronchopneumonia, focal, acute			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kidney			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gall Bladder			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary Gland			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sialadenitis, focal, chronic			3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mandibular Lymph Node			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Lymph Node			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bone Marrow			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diaphragm			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large Intestine			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

## APPENDIX B

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aAverage total hydrocarbon concentration of DF2 exhaust =  $0.006 + 0.006 \text{ mg/l}$

<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 + 0.450 \text{ mg/l}$

## APPENDIX B

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Table B-7. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smoke Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smoke Exhaust (60 min)			
		00	01	02	03	00	01	02	03	00	01	02	03	00	01	02	03	00	01	02	03
Bone Marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic		2	1	1	1	1	2	1	1	2	1	1	1	1	2	1	1	1	2	2	1
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, focal, chronic																					
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turbinates		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, multifocal																					
Hemorrhage, focal																					
Congestion, diffuse																					
Congestion, multifocal																					
Congestion, focal																					
Skin																					
Testes		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypoplasia, unilateral																					
Mammary Gland		-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Urinary Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calculus		P	P																		
Seminal Vesicle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cervical Lymph Node		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Larynx		-	-	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+

Exposure: 32 days

Post exposure: 0 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust = 0.006 ± 0.006 mg/l<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust = 2.340 ± 0.450 mg/l

\*Animal died spontaneously 5 days after 32 exposures to DF2 smoke/exhaust. Death was ruled not due to exposure.



Table B-8. Histopathological Effects in Male Fischer 344 Rats After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Animal No.	No. of exposure days	Air Controls (60 min)										DF2 Smokeb Exhaust (15 min)										DF2a Exhaust (60 min)										DF2 Smokeb Exhaust (60 min)											
		0001-0005	0006-0010	0011-0015	0016-0020	0021-0025	0026-0030	0031-0035	0036-0040	0041-0045	0046-0050	0051-0055	0056-0060	0061-0065	0066-0070	0071-0075	0076-0080	0081-0085	0086-0090	0091-0095	0096-0100	0101-0105	0106-0110	0111-0115	0116-0120	0121-0125	0126-0130	0131-0135	0136-0140	0141-0145	0146-0150	0151-0155	0156-0160	0161-0165	0166-0170	0171-0175	0176-0180	0181-0185	0186-0190	0191-0195	0196-0200		
Spinal Cord		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Brain		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Eye		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pituitary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Esophagus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adrenals		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Parathyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymphocytosis, peribronchiolar, multifocal		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Peribronchiolitis, multifocal, chronic, active		1																																									
Peribronchiolitis, focal, chronic, active																																											
Peribronchiolitis, multifocal, chronic																																											
Peribronchiolitis, focal, chronic																																											
Congestion, diffuse		3	2	2																																							
Kidney		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Nephropathy, multifocal, chronic		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver																																											
Hepatitis, periportal, multifocal, chronic																																											
Biliary cyst, solitary																																											
Malformation, Supernumerary lobe		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Congestion, diffuse																																											
Salivary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Atrophy, focal																																											

Table B-8. (Continued)

Table B-8. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)			DF2a Exhaust (15 min)			DF2 Smoke Exhaust (15 min)			DF2a Exhaust (60 min)			DF2 Smoke Exhaust (60 min)		
		001005	0021005	0031005	004005	005005	006005	007005	008005	009005	010005	011005	012005	013005	014005	015005
Mandibular Lymph Node		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Lymph Node		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bone Marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic		12121	1	2	1	2	1	1	1	1	11	21	12	1	321	21
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, focal, chronic																
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematodiasis		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Turbinates		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, multifocal																
Hemorrhage, focal																
Congestion, diffuse		2														
Congestion, multifocal		1														
Congestion, focal																
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypoplasia, unilateral																
Mammary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calculus		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Seminal Vesicle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian Gland		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Exposure: 65 days																
Post exposure: 0 days																

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust = 0.006 ± 0.006 mg/l<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust = 2.340 ± 0.450 mg/l

\*Sacrificed for correlation of physiological and pathological evaluations.

\*\*Spontaneous death. Cause of death not due to exposure.

Table B-9. Histopathological Effects in Male Fischer 344 Rats after Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

	No. of exposure days	Animal No.	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smokeb Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smokeb Exhaust (60 min)			
			01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04
Spinal Cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Peribronchiolitis, multifocal, chronic																						
Histiocytosis, focal																						
Congestion, diffuse																						
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nephropathy, multifocal, chronic	1	2																				
Lymphocytosis, interstitial, multifocal																						
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatitis, periportal, multifocal, chronic																						
Focal Cellular Alteration																						
Necrosis, focal																						
Microabscess, multiple																						
Microabscess, solitary																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary Gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mandibular Lymph Node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Lymph Node	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bone Marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table B-9. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)						DF2a Exhaust (15 min)						DF2 Smoke Exhaust (15 min)						DF2a Exhaust (60 min)						DF2 Smoke Exhaust (60 min)					
		01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic	1	1	2	1																											
Myocarditis, focal, chronic																															
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, focal, chronic																															
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematodiasis																															
Turbinates		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, diffuse																															
Hemorrhage, focal																															
Congestion, diffuse																															
Congestion, focal																															
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypoplasia, diffuse																															
Mammary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calculus																															
Seminal Vesicle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Exposure: 65 days  
Post exposure: 30 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l

<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l

\*Spontaneous death. Cause not related to exposure.

\*\*Sacrificed for correlation of physiological and pathological evaluations.

APPENDIX B

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Table B-10. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)				DF2a Exhaust (15 min)				DF2 Smoke Exhaust (15 min)				DF2a Exhaust (60 min)				DF2 Smoke Exhaust (60 min)			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic		1	1																		
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, multifocal, chronic																					
Pancreatitis, focal, chronic																					
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turbinates		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, multifocal																					
Hemorrhage, focal																					
Congestion, diffuse																					
Congestion, multifocal		1	1	2	2																
Congestion, focal																					
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mammary Gland		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal Muscle		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemorrhage, focal																					

Exposure: 32 days  
Post exposure: 0 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l

<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l

Table B-11. Histopathological Effects in Female Fischer 344 Rats After Airborne Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Animal No.	No. of exposure days	Air Controls (60 min)					DF2a Exhaust (15 min)					DF2 Smokeb Exhaust (15 min)					DF2a Exhaust (60 min)					DF2 Smokeb Exhaust (60 min)						
		00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000
Spinal Cord		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brain		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eye		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Larynx		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphocytosis, peribronchiolar, multifocal		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Peribronchiolitis, multifocal, chronic																												
Peribronchiolitis, focal, chronic																												
Histiocytosis, focal																												
Congestion, diffuse		1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kidney		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatitis, periportal, multifocal, chronic																												
Hyperplasia, bile duct, focal																												
Accessory Lobe, congenital																												
Spleen		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastular lymph Node		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric lymph Node		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bone Marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myocarditis, multifocal, chronic		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table B-11. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)						DF2a Exhaust (15 min)						DF2 Smoke Exhaust (15 min)						DF2a Exhaust (60 min)						DF2 Smoke Exhaust (60 min)					
		0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000
Thigh Muscle		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sciatic Nerve		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parotids		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, focal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematodiasis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tuberculosis		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, focal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion, diffuse		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion, multifocal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion, focal		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parovarian Cyst, unilateral		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrometra, diffuse		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mammary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vagina		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Exposure: 65 days

Post exposure: 0 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust = 0.006 ± 0.006 mg/l<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust = 2.340 ± 0.450 mg/l

\*Sacrificed for correlation of physiological and pathological evaluations.

\*\*Male accessory organs included - tissue processing error.



APPENDIX B

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Table B-12. (Continued)

Animal No.	No. of exposure days	Air Controls (60 min)					DF2a Exhaust (15 min)					DF2 Smoke Exhaust (15 min)					DF2a Exhaust (60 min)					DF2 Smoke Exhaust (60 min)				
		01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234	01234
Sciatic Nerve		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatitis, multifocal, chronic																										
Pancreatitis, focal, chronic																										
Large Intestine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematodiasis																										
Turbinates		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemorrhage, focal																										
Congestion, focal																										
Skin		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Farovarian Cyst, unilateral																										
Uterus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mammary Gland		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenteric Fat		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steatitis, focal, chronic, active																										

Exposure: 65 days

Post exposure: 30 days

<sup>a</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l

APPENDIX C

CLIMATIC CONDITIONS OF ANIMAL EXPOSURE CHAMBER DURING STUDIES TO DETERMINE  
THE SUBCHRONIC INHALATION TOXICITY OF M60A1 TANK-GENERATED DF2  
(DIESEL FUEL) SMOKE AND/OR EXHAUST

Blank

Table C-1. Animal Exposure Chamber Climatic Conditions During Studies Designed to Determine the Subchronic (Inhalation) Toxicity of M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust (August)

MONTH	CONDITION	CLIMATIC PARAMETER MEASURED										Time After Exposure Chamber Filling (Minutes)			
		0	1	5	10	15	30	60							
August	Mock	a/ b/ 76.9±4.3	a/ b/ 78.5±1.8	a/ b/ 79.0±1.4	a/ b/ 78.0±0.0	a/ b/ 77.6±1.7	a/ b/ 76.5±4.2								
	Control	a/ b/ 74.3±4.3	a/ b/ 78.3±2.7	a/ b/ 78.0±2.8	a/ b/ 83.0±0.0	a/ b/ 79.2±2.8	a/ b/ 78.0±4.0								
	(60 min.)	a/ b/ 86.9±6.0	a/ b/ 88.7±5.4	a/ b/ 92.0±5.7	a/ b/ 76.0±0.0	a/ b/ 85.6±5.3	a/ b/ 86.9±3.1								
	Ambient Relative Humidity (%)	a/ b/ 81.6±7.2	a/ b/ 86.8±5.4	a/ b/ 84.5±2.1	a/ b/ 73.0±0.0	a/ b/ 84.0±7.9	a/ b/ 82.0±4.3								
	DF2	a/ b/ 75.8±3.6	a/ b/ 76.1±3.6	a/ b/ 76.2±3.6	a/ b/ 76.2±3.8	a/ b/ 76.3±3.6									
	Exhaust	a/ b/ 78.1±4.3	a/ b/ 79.4±3.3	a/ b/ 79.4±3.1	a/ b/ 79.0±3.2	a/ b/ 79.1±3.0									
	(15 min.)	a/ b/ 87.0±2.8	a/ b/ 86.0±3.8	a/ b/ 86.1±3.7	a/ b/ 86.1±4.2	a/ b/ 86.1±4.0									
	Ambient Relative Humidity (%)	a/ b/ 85.2±6.4	a/ b/ 88.5±4.3	a/ b/ 88.5±4.4	a/ b/ 88.2±4.4	a/ b/ 87.8±4.0									
	DF2	a/ b/ 75.8±3.6	a/ b/ 75.9±3.9	a/ b/ 76.0±3.8	a/ b/ 77.6±2.2	a/ b/ 75.9±4.0	a/ b/ 75.8±4.1								
	Exhaust	a/ b/ 78.4±3.7	a/ b/ 79.3±3.2	a/ b/ 79.3±3.4	a/ b/ 80.1±1.9	a/ b/ 79.1±3.5	a/ b/ 80.2±5.1								
	(60 min.)	a/ b/ 86.8±4.1	a/ b/ 86.4±3.8	a/ b/ 86.3±3.5	a/ b/ 87.1±4.5	a/ b/ 86.4±3.9	a/ b/ 86.0±3.1								
	Ambient Relative Humidity (%)	a/ b/ 91.9±5.5	a/ b/ 93.0±4.6	a/ b/ 92.8±4.8	a/ b/ 93.0±5.8	a/ b/ 90.7±4.0	a/ b/ 90.4±4.4								
	DF2	a/ b/ 75.2±4.2	a/ b/ 75.0±3.7	a/ b/ 74.8±4.1	a/ b/ 75.2±3.5	a/ b/ 74.9±3.9									
	Smoke/	a/ b/ 77.6±4.3	a/ b/ 78.1±4.6	a/ b/ 78.1±4.7	a/ b/ 78.4±4.2	a/ b/ 78.3±4.1									
	Exhaust	a/ b/ 86.0±4.0	a/ b/ 85.0±3.8	a/ b/ 84.9±3.9	a/ b/ 85.7±3.9	a/ b/ 85.6±4.0									
	Ambient Relative Humidity (%)	a/ b/ 91.4±3.4	a/ b/ 91.7±2.7	a/ b/ 91.5±3.4	a/ b/ 91.8±3.6	a/ b/ 91.0±3.3									
	DF2	a/ b/ 74.8±4.1	a/ b/ 74.7±4.2	a/ b/ 74.6±4.8	a/ b/ 74.6±4.3		a/ b/ 74.8±4.2	a/ b/ 74.2±3.9							
	Smoke/	a/ b/ 78.1±4.3	a/ b/ 78.7±3.8	a/ b/ 78.4±4.2	a/ b/ 78.3±3.8		a/ b/ 77.8±4.6	a/ b/ 77.8±4.4							
	Exhaust	a/ b/ 86.1±3.8	a/ b/ 85.6±4.1	a/ b/ 84.5±4.1	a/ b/ 85.3±4.4		a/ b/ 86.3±4.0	a/ b/ 87.0±3.5							
	Ambient Relative Humidity (%)	a/ b/ 90.0±4.9	a/ b/ 91.6±4.0	a/ b/ 91.3±3.4	a/ b/ 91.1±4.2		a/ b/ 88.2±3.5	a/ b/ 87.8±3.7							

NOTE: a/ Mean; b/ Standard Deviation

Table C-2. Animal Exposure Chamber Climatic Conditions During Studies Designed to Determine the Subchronic (Inhalation) Toxicity of M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust (September)

MONTH	CONDITION	CLIMATIC PARAMETER MEASURED	Time After Exposure Chamber Filling (Minutes)					
			0	1	5	10	15	30
September	Mock	Ambient Temperature (°F)	a/ b/ 70.6±5.5	a/ b/ 68.5±4.2	a/ b/ 77.8±4.0	a/ b/ 68.6±6.3	a/ b/ 73.4±5.1	a/ b/ 70.3±5.8
	Control	Chamber Temperature (°F)	71.8±4.3	73.5±4.0	78.3±2.6	72.9±5.1	77.6±3.6	74.4±4.6
	(60 min.)	Ambient Relative Humidity (%)	75.6±7.7	73.7±8.2	77.8±5.3	73.0±9.2	79.4±6.1	75.2±7.0
		Exposure Chamber Relative Humidity (%)	83.3±7.4	81.3±6.7	90.8±1.7	82.0±7.4	87.0±5.7	81.7±6.8
	DF2	Ambient Temperature (°F)	69.8±5.7	69.6±5.9	69.6±5.8	69.8±5.4	70.2±6.1	-----
	Exhaust	Exposure Chamber Temperature (°F)	72.8±3.8	75.2±3.8	75.0±4.0	74.6±3.9	74.5±3.9	-----
	(15 min.)	Ambient Relative Humidity (%)	77.1±7.3	76.3±7.9	76.3±7.9	76.0±7.8	75.5±8.2	-----
		Exposure Chamber Relative Humidity (%)	79.4±6.2	80.4±7.3	80.8±7.3	80.8±7.7	80.6±7.6	-----
	DF2	Ambient Temperature (°F)	68.6±5.2	68.6±5.1	68.6±5.1	69.0±5.1	-----	67.8±5.4
	Exhaust	Exposure Chamber Temperature (°F)	71.5±4.2	74.1±3.7	74.0±3.5	74.4±3.4	-----	73.7±4.6
	(60 min.)	Ambient Relative Humidity (%)	76.1±8.1	76.3±7.6	76.2±7.8	76.2±7.7	-----	75.9±8.8
		Exposure Chamber Relative Humidity (%)	80.2±7.9	81.7±9.0	81.7±8.8	83.1±8.0	-----	80.8±8.1
	DF2	Ambient Temperature (°F)	68.1±5.7	68.1±5.7	67.5±5.2	68.0±5.8	68.0±5.8	-----
	Smoke/	Exposure Chamber Temperature (°F)	71.1±4.6	73.3±3.8	73.1±3.6	73.1±4.7	72.9±4.2	-----
	Exhaust	Ambient Relative Humidity (%)	76.6±9.6	76.6±9.3	76.2±9.4	76.5±9.3	76.3±9.5	-----
	(15 min.)	Exposure Chamber Relative Humidity (%)	80.7±9.9	81.9±10.4	81.0±10.0	81.4±10.2	82.1±10.0	-----
	DF2	Ambient Temperature (°F)	67.8±5.7	67.7±5.6	67.6±5.7	67.7±5.8	-----	67.3±6.0
	Smoke/	Exposure Chamber Temperature (°F)	70.7±4.7	73.0±4.1	72.8±4.1	72.9±4.3	-----	72.6±4.1
	Exhaust	Ambient Relative Humidity (%)	76.2±9.7	76.0±9.3	76.1±9.6	76.8±9.4	-----	77.0±9.5
	(60 min.)	Exposure Chamber Relative Humidity (%)	80.2±10.5	80.8±10.4	80.7±10.6	80.8±10.3	-----	81.1±9.2

LEGEND: a/ Mean; b/ Standard Deviation

Table C-3. Animal Exposure Chamber Climatic Conditions During Studies Designed to Determine the Subchronic (Inhalation) Toxicity of M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust (October)

MONTH	CONDITION	CLIMATIC PARAMETER MEASURED	Time After Exposure Chamber Filling (Minutes)									
			0	1	5	10	15	30	60			
October	Mock	Ambient Temperature (°F)	a/ b/ 71.3±2.5	a/ b/ 70.4±5.7	a/ b/ 66.8±11.8	a/ b/ 66.8±10.5	a/ b/ -----	a/ b/ -----	a/ b/ 71.3±2.2			
	Control	Exposure Chamber Temperature (°F)	68.3±4.6	69.7±6.1	66.2±7.3	67.5±7.3	-----	-----	70.2±6.0			
	(60 min.)	Ambient Relative Humidity (%)	47.9±21.6	47.2±21.2	49.0±18.7	52.2±18.9	-----	-----	49.5±23.1			
		Exposure Chamber Relative Humidity (%)	66.4±15.4	65.3±14.4	59.6±10.9	58.3±10.0	-----	-----	68.2±15.5			
	DF2	Ambient Temperature (°F)	69.9±5.8	70.2±5.8	70.5±5.8	70.2±7.2	70.2±5.9	-----	-----			
	Exhaust	Exposure Chamber Temperature (°F)	69.1±4.9	70.9±4.9	70.6±4.8	70.6±5.7	70.6±5.0	-----	-----			
	(15 min.)	Ambient Relative Humidity (%)	51.8±22.7	50.6±22.0	50.8±22.4	52.9±23.2	51.2±22.6	-----	-----			
		Exposure Chamber Relative Humidity (%)	65.7±13.6	64.5±13.6	64.6±13.5	65.4±15.1	65.0±13.8	-----	-----			
	DF2	Ambient Temperature (°F)	71.8±2.8	70.7±6.0	71.0±6.1	70.9±7.0	-----	72.3±2.7	70.8±6.4			
	Exhaust	Exposure Chamber Temperature (°F)	68.8±2.7	70.8±5.3	70.5±5.1	70.3±5.8	-----	70.1±5.3	71.1±5.2			
	(60 min.)	Ambient Relative Humidity (%)	47.8±22.7	48.5±22.0	48.5±22.0	49.6±22.0	-----	51.7±24.5	49.2±22.9			
		Exposure Chamber Relative Humidity (%)	65.6±15.8	64.1±14.9	64.2±15.0	65.0±15.1	-----	68.9±16.2	64.8±14.9			
	DF2	Ambient Temperature (°F)	73.0±3.8	71.6±7.0	71.6±7.0	71.4±6.9	71.2±7.1	-----	-----			
	Smoke/	Exposure Chamber Temperature (°F)	70.6±4.3	71.1±5.4	71.1±5.4	71.0±5.5	70.4±5.3	-----	-----			
	Exhaust	Ambient Relative Humidity (%)	46.9±22.9	48.2±22.6	48.4±22.2	48.1±22.2	49.0±22.6	-----	-----			
	(15 min.)	Exposure Chamber Relative Humidity (%)	64.5±15.7	63.5±15.0	63.3±15.0	63.2±15.1	63.6±15.2	-----	-----			
	DF2	Ambient Temperature (°F)	72.5±3.8	71.4±6.9	71.4±6.9	71.0±7.1	-----	70.2±7.2	70.0±7.3			
	Smoke/	Exposure Chamber Temperature (°F)	69.5±3.9	70.6±5.9	70.6±6.2	70.1±6.0	-----	70.5±6.1	70.2±6.1			
	Exhaust	Ambient Relative Humidity (%)	46.8±22.5	47.7±22.7	47.7±22.9	47.4±22.9	-----	48.6±22.7	49.0±23.1			
	(60 min.)	Exposure Chamber Relative Humidity (%)	63.1±15.3	62.3±15.0	62.3±14.9	62.3±15.0	-----	62.5±14.9	62.9±14.9			

LEGEND: a/ Mean; b/ Standard Deviation

Table C-4. Animal Exposure Chamber Climatic Conditions During Studies Designed to Determine the Subchronic (Inhalation) Toxicity of M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust (November)

		Time After Exposure Chamber Filling (Minutes)							
		CLIMATIC PARAMETER MEASURED							
MOISTURE	CONDITION	0	1	5	10	15	30	60	
Control (60 min.)	Ambient Temperature (°F)	71.9±3.9	73.0±3.6	72.0±4.6	70.7±4.6	-----	68.0±0.0	75.1±3.4	
	Exposure Chamber Temperature (°F)	66.9±3.2	69.6±3.0	69.0±2.7	70.0±2.0	-----	70.0±0.0	71.0±2.5	
	Ambient Relative Humidity (%)	33.3±10.9	33.4±10.2	31.0±4.3	31.0±5.3	-----	36.0±0.0	32.8±10.8	
	Exposure Chamber Relative Humidity (%)	58.6±8.2	59.3±6.0	58.0±6.7	56.0±2.2	-----	57.0±0.0	59.0±8.0	
Exhaust (15 min.)	Ambient Temperature (°F)	73.6±3.8	73.3±3.5	73.7±3.4	73.5±3.5	73.7±3.3	-----	-----	
	Exposure Chamber Temperature (°F)	70.1±3.3	71.5±2.9	71.4±2.7	72.1±2.4	71.1±2.5	-----	-----	
	Ambient Relative Humidity (%)	32.6±10.5	32.7±10.5	32.9±11.0	33.5±11.9	33.0±11.2	-----	-----	
	Exposure Chamber Relative Humidity (%)	58.6±7.8	58.6±7.7	58.7±7.7	58.6±7.9	58.6±7.6	-----	-----	
Exhaust (60 min.)	Ambient Temperature (°F)	73.6±3.4	74.0±3.5	74.1±3.4	74.5±3.3	-----	74.4±3.4	74.0±3.5	
	Exposure Chamber Temperature (°F)	69.5±2.5	70.6±3.0	70.7±2.9	71.0±3.0	-----	71.6±3.0	72.0±3.0	
	Ambient Relative Humidity (%)	32.6±11.7	32.8±12.0	32.9±12.0	32.8±12.2	-----	32.8±12.6	32.9±13.3	
	Exposure Chamber Relative Humidity (%)	57.9±7.9	57.6±7.8	57.7±7.8	57.5±8.2	-----	58.4±8.2	58.0±8.6	
Smoke/ Exhaust (15 min.)	Ambient Temperature (°F)	74.6±4.0	75.1±3.4	74.5±4.0	74.1±4.1	73.8±4.0	-----	-----	
	Exposure Chamber Temperature (°F)	70.7±3.2	71.5±3.1	71.3±3.1	71.5±3.3	71.6±3.4	-----	-----	
	Ambient Relative Humidity (%)	33.1±12.7	32.6±12.8	33.2±12.8	33.4±12.3	33.9±12.6	-----	-----	
	Exposure Chamber Relative Humidity (%)	57.2±8.1	57.2±8.2	57.3±8.1	57.3±8.1	57.4±8.4	-----	-----	
Smoke/ Exhaust (60 min.)	Ambient Temperature (°F)	73.7±3.9	74.0±3.8	74.1±3.8	73.9±3.9	-----	73.5±4.1	73.5±4.3	
	Exposure Chamber Temperature (°F)	70.7±3.1	71.1±3.4	71.1±3.2	71.1±3.2	-----	71.3±3.6	71.5±3.6	
	Ambient Relative Humidity (%)	34.2±13.1	34.1±13.2	34.1±13.2	34.1±13.2	-----	34.3±12.9	34.5±13.5	
	Exposure Chamber Relative Humidity (%)	56.5±8.5	56.5±8.5	57.1±8.6	57.2±8.7	-----	57.6±8.8	58.6±9.4	

LEGEND: a/ Mean; b/ Standard Deviation



Table C-5. Animal Exposure Chamber Climatic Conditions During Studies Designed to Determine the Subchronic (Inhalation) Toxicity of M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust (December)

MONTH		CONDITION	CLIMATIC PARAMETER MEASURED												Time After Exposure Chamber Filling (Minutes)			
			0	1	5	10	15	30	60									
December	Mock	Ambient Temperature (°F)	70.6±3.4	71.4±3.9	a/ b/ 72.0±3.0 72.5±3.1	a/ b/ 72.5±3.1 72.6±2.8	a/ b/ 72.7±2.8	a/ b/ 72.0±3.1	a/ b/ 71.8±3.1									
	Control (60 min.)	Exposure Chamber Temperature (°F)	66.6±3.6	68.7±4.3	68.6±4.3	69.3±4.1	69.9±2.0	69.0±3.8	69.6±3.6									
		Ambient Relative Humidity (%)	46.5±3.8	47.0±3.8	47.3±3.4	47.5±3.6	47.5±3.6	47.4±3.5	46.7±3.5									
		Exposure Chamber Relative Humidity (%)	46.5±3.8	47.0±3.8	47.3±3.4	47.5±3.6	47.5±3.6	47.4±3.5	46.7±3.5									
	DF2	Ambient Temperature (°F)	72.1±3.1	72.7±3.2	72.5±3.1	72.6±2.8	72.7±2.8	72.7±2.8	72.7±2.8									
	Exhaust (15 min.)	Exposure Chamber Temperature (°F)	68.8±2.9	70.0±2.6	69.8±2.7	70.4±2.4	69.9±2.0	69.9±2.0	69.9±2.0									
		Ambient Relative Humidity (%)	22.4±5.0	22.6±5.3	22.5±5.0	22.6±5.0	22.5±5.0	22.5±5.0	22.5±5.0									
		Exposure Chamber Relative Humidity (%)	46.6±3.4	46.8±3.2	47.0±2.7	47.1±2.7	47.2±2.7	47.2±2.7	47.2±2.7									
	DF2	Ambient Temperature (°F)	72.9±3.1	73.0±3.1	72.9±3.3	73.0±3.1	73.3±3.1	73.3±3.1	74.2±2.7									
	Exhaust (60 min.)	Exposure Chamber Temperature (°F)	67.8±3.1	69.5±2.4	69.7±2.3	69.5±2.2	70.1±1.7	70.1±1.7	71.3±1.2									
		Ambient Relative Humidity (%)	22.4±4.7	22.4±4.7	22.4±4.6	22.4±4.7	22.4±4.4	22.4±4.4	21.8±4.6									
		Exposure Chamber Relative Humidity (%)	47.0±2.4	47.3±2.6	47.4±2.6	47.4±2.6	47.4±2.6	47.4±2.6	47.4±2.6									
	DF2	Ambient Temperature (°F)	74.4±2.8	74.6±3.0	74.7±3.0	74.4±3.2	73.9±3.2	73.9±3.2	73.9±3.2									
	Smoke/Exhaust (15 min.)	Exposure Chamber Temperature (°F)	69.9±3.4	70.8±2.3	70.9±2.1	70.7±2.5	70.4±2.0	70.4±2.0	70.4±2.0									
		Ambient Relative Humidity (%)	21.5±4.6	21.6±4.9	21.7±5.1	21.6±4.9	21.7±4.8	21.7±4.8	21.7±4.8									
		Exposure Chamber Relative Humidity (%)	47.2±2.4	47.1±2.4	47.1±2.4	47.1±2.4	47.2±2.4	47.2±2.4	47.2±2.4									
	DF2	Ambient Temperature (°F)	70.5±4.0	71.6±3.6	72.6±3.0	72.6±3.1	72.8±2.7	72.8±2.7	73.7±3.1									
	Smoke/Exhaust (60 min.)	Exposure Chamber Temperature (°F)	67.9±3.9	69.9±2.4	70.1±2.6	70.3±2.5	70.5±1.7	70.5±1.7	71.2±2.2									
		Ambient Relative Humidity (%)	22.6±5.4	22.3±5.2	22.5±5.2	22.5±5.2	22.4±5.5	22.4±5.5	21.9±4.9									
		Exposure Chamber Relative Humidity (%)	47.1±2.4	47.1±2.4	47.2±2.5	47.2±2.5	47.2±2.5	47.2±2.5	47.3±2.5									

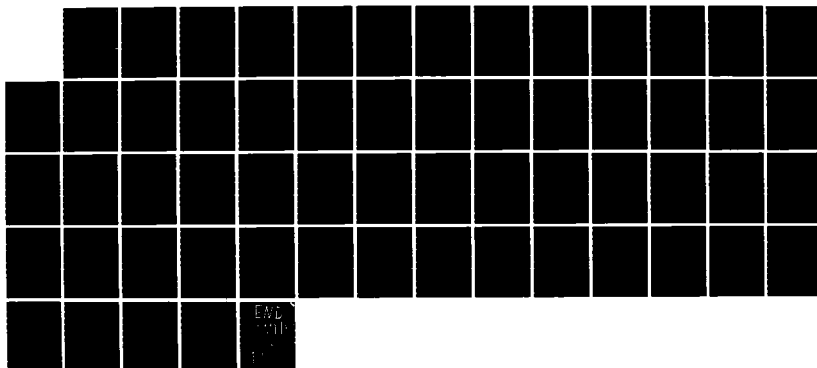
LEGEND: a/ Mean; b/ Standard Deviation

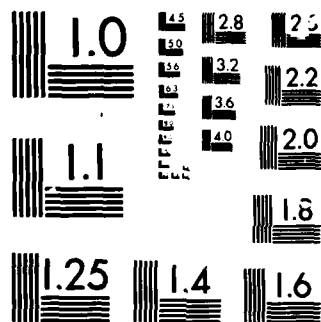
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THE SUBCHRONIC INHALATION TOXICITY OF DF2 (DIESEL FUEL) 2/2  
USED IN VEHICLE E. (U) CHEMICAL RESEARCH AND  
DEVELOPMENT CENTER ABERDEEN PROVING GRO..  
J F CALLAHAN ET AL. MAR 86 CRDC-TR-85009 F/G 6/28 NL

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APPENDIX D

THE GEOMETRIC MEAN DIAMETER OF CARBONACEOUS PARTICLES FOUND IN  
CLOUDS OF DF2 (DIESEL FUEL) SMOKE/EXHAUST GENERATED  
BY THE M60A1 TANK

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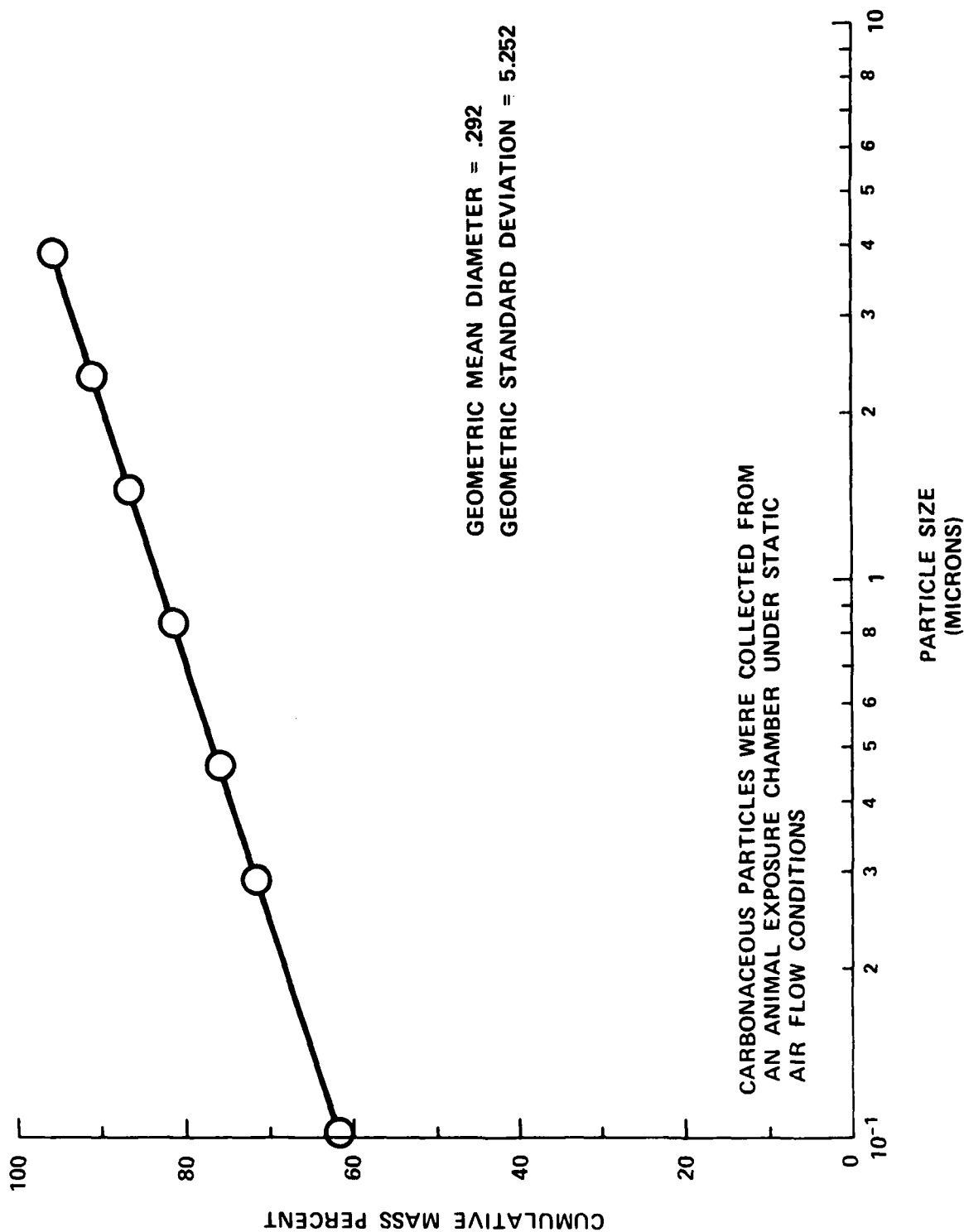


Figure D-1. The Geometric Mean Diameter of Carbonaceous Particles Found in Clouds of DF2 (Diesel Fuel) Smoke/Exhaust Generated by the M60A1 Tank

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APPENDIX E

THE GROWTH RATES OF B6C3F1 MICE AND FISCHER 344 RATS EXPOSED  
BY THE AIRBORNE ROUTE TO DF2 (DIESEL FUEL)  
SMOKE AND/OR EXHAUST GENERATED BY THE M60A1 TANK

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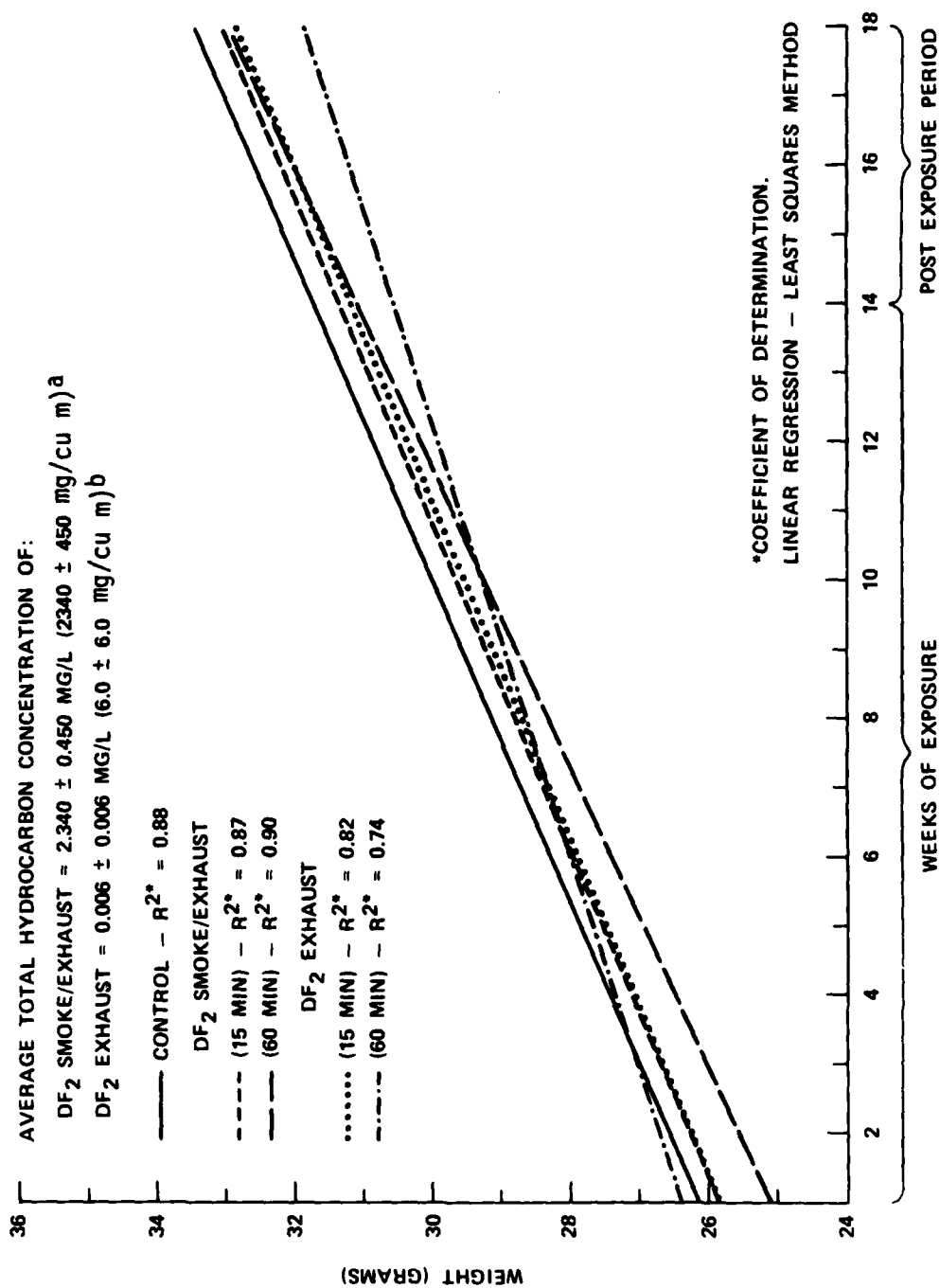


Figure E-1. Growth Rates in Male B6C3F<sub>1</sub> Mice Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

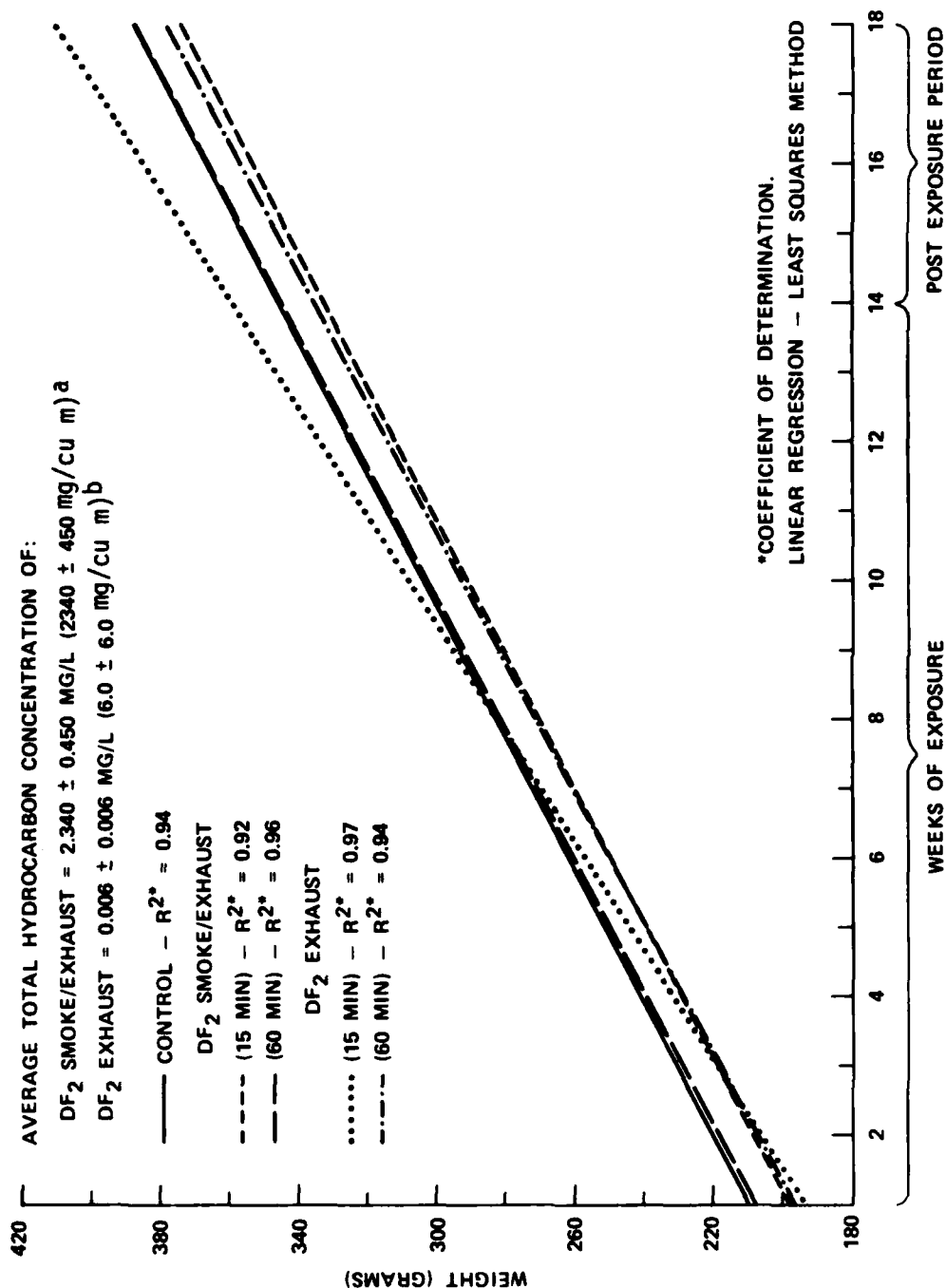


Figure E-2. Growth Rates in Female B6C3F1 Mice Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

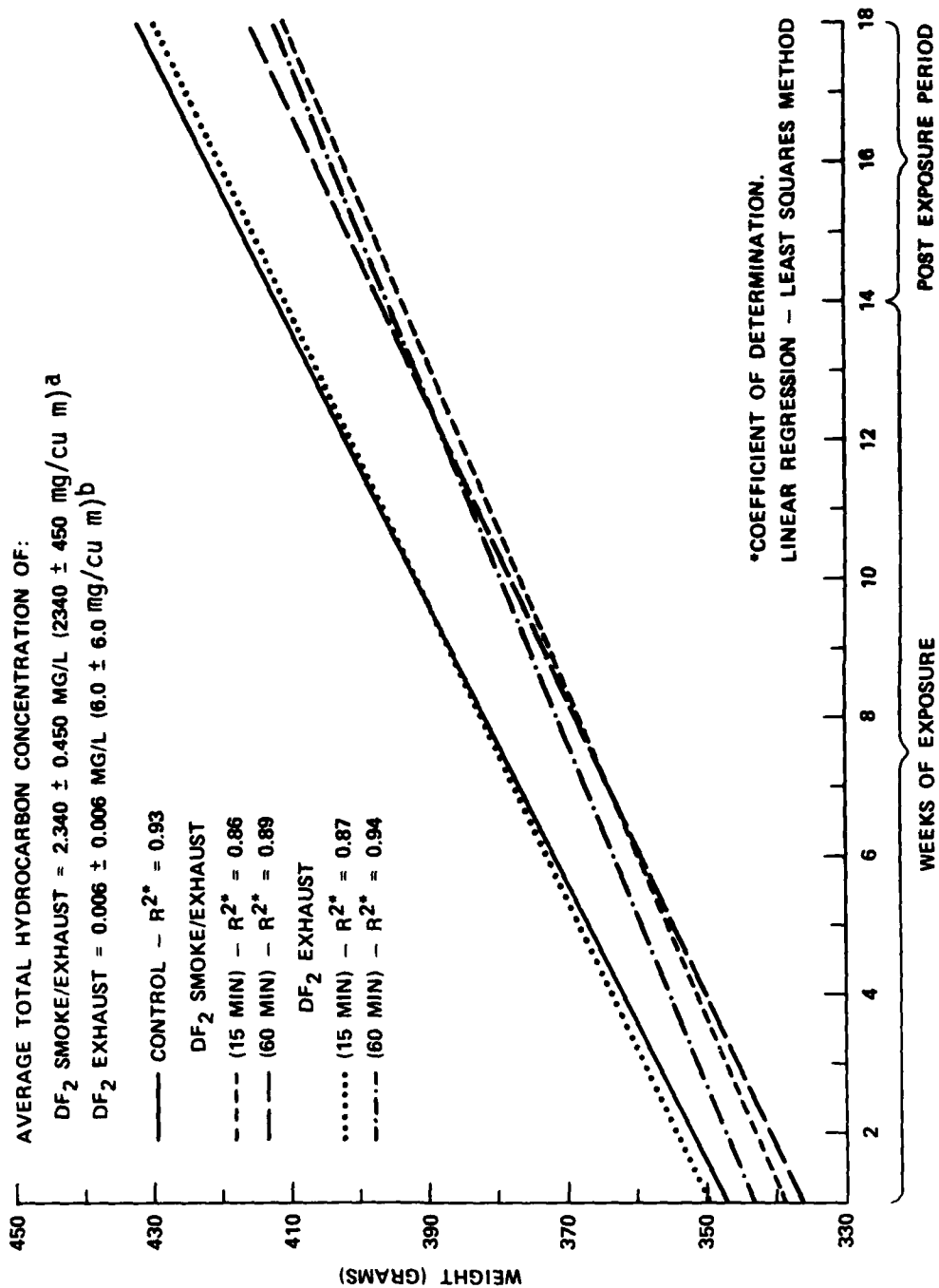


Figure E-3. Growth Rates in Male Fischer 344 Rats (13 Weeks Old) Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

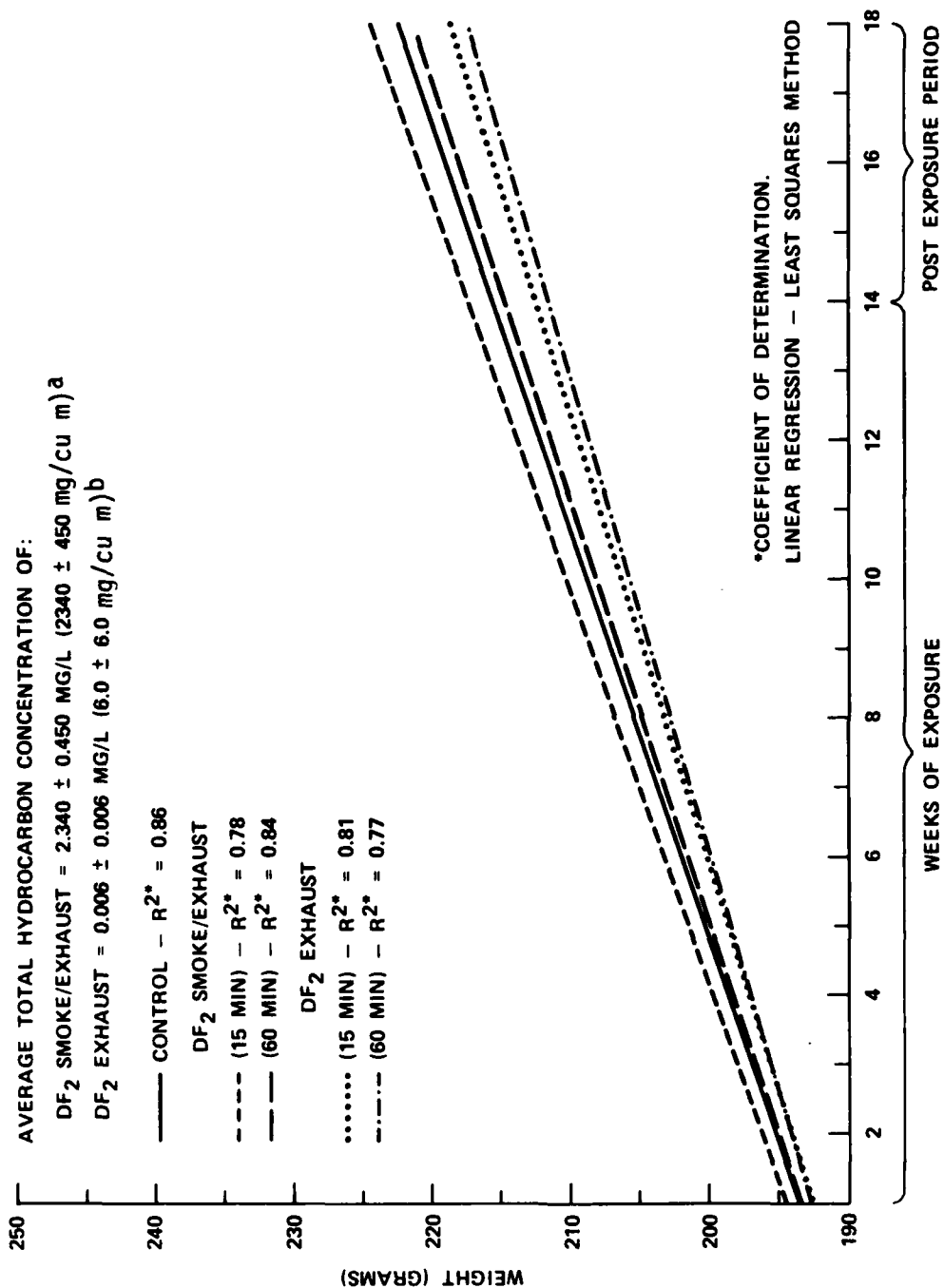


Figure E-4. Growth Rates in Female Fischer 344 Rats (13 Weeks Old) Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

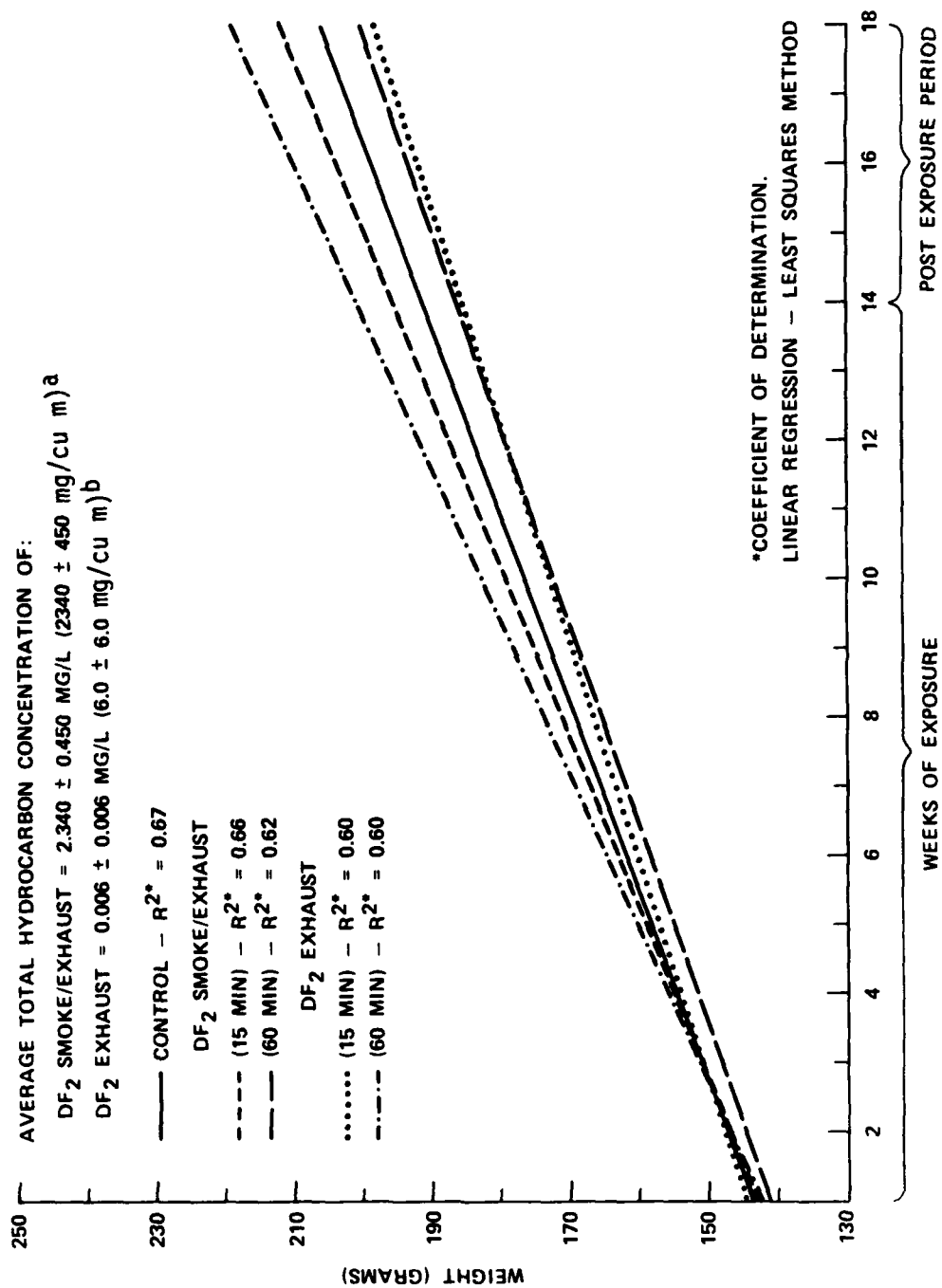


Figure E-5. Growth Rates in Male Fischer 344 Rats (10 Weeks Old) Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

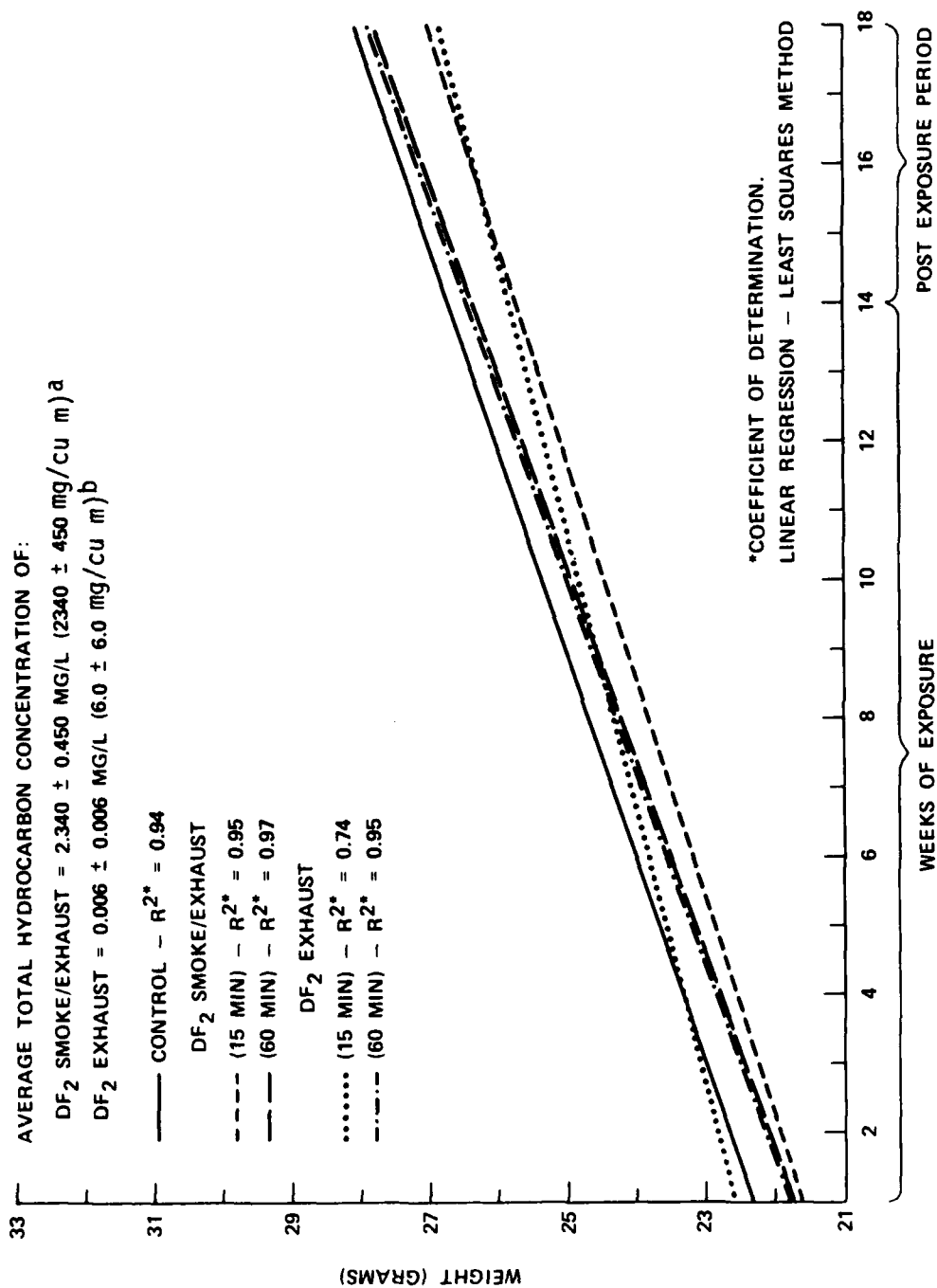


Figure E-6. Growth Rates in Female Fischer 344 Rats (10 Weeks Old) Exposed by the Airborne Route to M60A1 Tank-Generated DF<sub>2</sub> (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>



APPENDIX F

HEMATOLOGICAL AND BLOOD CHEMICAL EFFECTS IN B6C3F1 MICE AND  
FISCHER 344 RATS AFTER EXPOSURE BY THE AIRBORNE ROUTE  
TO DF2 (DIESEL FUEL) SMOKE AND/OR EXHAUST  
GENERATED BY THE M60A1 TANK

Blank

Table F-1. Units of Measurement Used During the Hematological and Blood Chemical Evaluation of B6C3F1 Mice and Fischer 344 Rats After Airborne Exposure to DF2 (Diesel Fuel) Smoke and/or Exhaust.

<u>Symbol</u>	<u>Nomenclature</u>	<u>Units of Measurement</u>
RBC	Red blood cells	106/cu mm
WBC	White blood cells	Per cu mm
HCT	Hematocrit	%
HGB	Hemoglobin	mg/dl
NEU	Neutrophils	%
BND	Band cells	%
LYM	Lymphocytes	%
MON	Monocytes	%
EOS	Eosinophils	%
BAS	Basophils	%
GLS	Glucose	mg/dl
UN	Urea nitrogen	mg/dl
CRT	Creatinine	mg/dl
NA	Sodium	meq/l
K	Potassium	meq/l
CL	Chloride	meq/l
CD	Carbon dioxide	meq/l
UA	Uric acid	mg/dl
TP	Total protein	g/dl
AN	Albumin	g/dl
GLN	Globulin	g/dl
CA	Calcium	mg/dl
CHL	Cholesterol	mg/dl
TRI	Triglycerides	mg/dl
ALP	Alkaline phosphatase	u/l
GOT	Serum glutamic oxalacetic transaminase	u/l
GPT	Serum glutamic pyruvic transaminase	u/l
LDH	Lactic dehydrogenase	u/l
BRN	Total bilirubin	mg/dl

Table F-2. Hematological Effects on B6C3F1 Mice After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : MALE

DOSE : CTL

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
a 7.29	.21	5	3740.00	212.79	5	35.94	1.11	5	15.40	.31	5	10.50	1.06	2	---	---	---	---
13W b 6.88	.56	6	5150.00	1183.39	6	33.00	2.67	6	13.78	1.04	6	31.00	.00	1	---	---	---	---
17W c 6.53	.55	6	2966.67	1239.77	6	31.50	2.63	6	13.58	.84	6	---	---	---	---	---	---	---

DOSE : EXH-15M d

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W e 5.93	.47	6	2733.33	219.43	6	30.30	1.49	6	12.30	.92	6	---	---	---	---	---	---	---
13W f 6.83	.53	6	4366.67	825.86	6	33.15	2.50	6	15.72	.93	6	45.00	.00	1	---	---	---	---
17W g 6.79	.37	6	2200.00	524.40	6	32.62	1.67	6	13.85	.43	6	---	---	---	---	---	---	---

DOSE : EXH-60M d

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W h 7.33	.28	6	3550.00	379.88	6	36.60	1.34	6	15.95	.30	6	11.00	6.36	2	---	---	---	---
13W i 6.68	.44	6	4866.67	867.84	6	31.93	2.28	6	13.72	.98	6	16.33	4.53	3	---	---	---	---
17W j 6.39	.32	6	3066.67	969.15	6	30.83	1.53	6	12.92	.72	6	---	---	---	---	---	---	---

'----' : INDICATE MISSING DATA

## LEGEND:

a 32-day (6-week) exposure period.

b 65-day (13-week) exposure period.

c 30-day (4-week) post-exposure period.

d Daily exposure period in minutes.

e Significant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P < 0.05$ \* Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-3. Hematological Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : MALE

DOSE : CTL

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	81.33	1.97	6	1.00	.00	2	1.33	.27	3	---	---	---
13W	73.50	4.88	4	---	---	---	---	---	---	---	---	---
17W	78.17	1.07	6	1.50	.35	2	1.00	.00	2	---	---	---

DOSE : EXH-15M d

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	71.17	2.99	6	2.50	.35	2	1.00	.00	3	---	---	---
13W	73.33	.93	6	---	---	---	1.00	.00	1	---	---	---
17W	71.00	3.53	6	1.00	.00	1	2.00	.00	3	---	---	---

DOSE : EXH-60M d

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	66.33	4.76	6	4.00	.71	2	3.33	.27	3	---	---	---
13W	82.00	.94	4	---	---	---	---	---	---	---	---	---
17W	68.67	2.64	6	1.33	.27	3	1.33	.27	3	---	---	---

--- : INDICATE MISSING DATA

#### LEGEND:

a32 day (6 week) exposure period.  
 b65 day (13 week) exposure period.  
 c30 day (4 week) post-exposure period.  
 dDaily exposure period in minutes.  
 eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P \leq 0.05$

\*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-4. Hematological Effects on B6C3F1 Mice After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W a	6.55	.43	6	4883.33	686.14	6	37.07	2.22	6	13.87	.65	6	25.50	1.94	6	---	---	---
13W b	6.52	.39	6	4666.67	1054.27	6	35.78	2.09	6	13.92	.86	6	31.75	4.77	4	---	---	---
17W c	6.16	.45	6	5450.00	468.89	6	33.90	2.70	6	13.75	.93	6	21.17	3.05	6	---	---	---

DOSE : EXH-15M d

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	6.17	.27	5	4860.00	507.23	5	34.62	1.79	5	13.60	.45	5	20.75	4.68	4	---	---	---
13W	7.25	.06	6	4216.67	500.79	6	39.60	.60	6	15.70	.16	6	22.00	6.19	5	---	---	---
17W	6.70	.50	6	5750.00	829.57	6	36.70	2.70	6	14.75	1.02	6	18.50	2.71	6	---	---	---

DOSE : EXH-60M d

TIME	RBC (10E6)			WBC			HCT			HGB			NEU			BND		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	6.79	.32	6	5600.00	913.48	6	37.55	1.89	6	15.13	.34	6	15.00	2.65	5	---	---	---
13W	6.92	.15	6	7716.67	3372.88	6	38.20	.75	6	14.53	.26	6	22.67	2.89	3	---	---	---
17W	6.93	.09	6	7250.00	1260.68	6	37.50	.81	6	15.27	.21	6	22.67	2.56	6	---	---	---

'---' : INDICATE MISSING DATA

## LEGEND:

- a32 day (6 week) exposure period.
- b65 day (13 week) exposure period.
- c30 day (4 week) post-exposure period.
- dDaily exposure period in minutes.
- eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P < 0.05$

\*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-5. Hematological Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	74.00	1.90	6	1.00	1.00	.00	1	1.00	.00	2	---	---
13W	68.00	4.95	4	---	---	---	---	---	.00	1	---	---
17W	76.83	3.10	6	1.00	1.00	.00	4	2.00	.61	4	---	---

DOSE : EXH-15M d

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	78.00	4.12	4	2.00	.71	2	1.00	.00	1	---	---	---
13W	78.00	6.19	5	---	---	---	---	---	---	---	---	---
17W	79.67	2.59	6	1.00	.00	2	1.80	.18	5	---	---	---

DOSE : EXH-60M d

TIME	LYM			MON			EOS			BAS		
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	84.40	2.51	5	1.00	.00	1	1.00	.00	2	---	---	---
13W	77.33	2.88	3	---	---	---	---	---	---	---	---	---
17W	76.17	2.42	6	1.00	.00	1	2.00	.47	3	---	---	---

'---' : INDICATE MISSING DATA

## LEGEND:

a32 day (6 week) exposure period.  
 b65 day (13 week) exposure period.  
 c30 day (4 week) post-exposure period.  
 dDaily exposure period in minutes.  
 eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P < 0.05$

\*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l  
 ( $6.0 \pm 6.0$  mg/cu m).

Table F-6. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : MALE

DOSE : CTL

TIME	CD		UA		TP		AN		GLN		CA	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W	22.67	1.19	1.08	.19	6.27	.11	3.55	.05	2.72	.12	10.70	.12
13W	23.33	2.04	1.20	.17	5.93	.13	3.53	.05	2.40	.11	10.28	.16
17W	24.67	.99	1.33	.37	6.33	.04	3.53	.04	2.80	.03	10.57	.11

DOSE : EXH-15M d

TIME	CD		UA		TP		AN		GLN		CA	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W	24.00	1.00	1.20	.17	6.37	.14	3.60	.07	2.77	.13	10.78	.08
13W	26.17	.50	1.20	.27	5.95	.09	3.45	.04	2.50	.07	10.23	.11
17W	25.83	1.16	.82	.09	6.22	.12	3.48	.05	2.73	.09	10.50	.17

DOSE : EXH-60M d

TIME	CD		UA		TP		AN		GLN		CA	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W	22.50	1.47	1.20	.22	6.23	.09	3.45	.06	2.78	.10	10.48	.13
13W	24.00	1.62	1.22	.19	6.15	.08	3.53	.04	2.62	.07	10.32	.14
17W	25.33	.69	1.98	.90	5.83	.35	7.90 e	4.04	6.63 e	3.55	10.52	.14

## LEGEND:

a32 day (6 week) exposure period.

b65 day (13 week) exposure period.

c30 day (4 week) post-exposure period.

dDaily exposure period in minutes.

eSignificant difference. Statistical evaluation included the Analysis of Variance and the

Student's "t" test.  $P \leq 0.05$ \*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).



Table F-7. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : FEMALE

DOSE : CTL																		
TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	200.83	21.39	6	19.50	.57	6	.72	.06	6	145.17	.72	6	5.27	.53	6	101.50	1.15	6
13W	178.67	18.38	6	19.33	.61	6	.62	.07	6	146.00	.59	6	5.08	.65	6	101.50	.51	6
17W	171.67	5.56	6	22.50	.84	6	.60	.06	6	146.33	.45	6	4.13	.17	6	101.67	.61	6
DOSE : EXH-15M d																		
TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	205.20	23.12	5	18.80	.59	5	.56	.08	5	145.40	.54	5	5.18	.85	5	102.80	.87	5
13W	207.67	27.54	6	20.50	.51	6	.53	.07	6	145.33	.30	6	5.42	.76	6	100.83	.64	6
17W	198.67	16.50	6	21.83	.50	6	.62	.04	6	146.00	.78	6	4.47	.50	6	101.83	.64	6
DOSE : EXH-60M d																		
TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	159.67	7.41	6	18.00	.75	6	.53	.03	6	145.67	.61	6	3.85	.07	6	102.83	.37	6
13W	176.33	7.30	6	19.83	.68	6	.60	.05	6	146.00	1.18	6	4.65	.15	6	103.00	.75	6
17W	186.83	11.53	6	21.83	.96	6	.58	.05	6	146.50	.46	6	5.00	.47	6	102.50	.39	6

## LEGEND:

a32 day (6 week) exposure period.

b65 day (13 week) exposure period.

c30 day (4 week) post-exposure period.

dDaily exposure period in minutes.

eSignificant difference. Statistical evaluation included the Analysis of Variance and the

Student's "t" test.  $P < 0.05$ \*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-8. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W <sup>a</sup>	18.00	1.49	6	2.20	.28	6	6.75	.11	6	3.78	.08	6	2.97	.06	6	10.83	.17	6
13W <sup>b</sup>	17.83	1.16	6	2.10	.34	6	6.88	.08	6	4.20	.06	6	2.68	.06	6	10.75	.11	6
17W <sup>c</sup>	18.83	.98	6	1.58	.23	6	6.93	.19	6	4.00	.12	6	2.93	.08	6	10.90	.21	6

DOSE : EXH-15M d

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	16.80	1.18	5	2.36	.58	5	6.50	.10	5	3.76	.14	5	2.74	.09	5	10.92	.19	5
13W	18.83	1.14	6	2.28	.70	6	6.80	.19	6	4.20	.17	6	2.60	.14	6	10.72	.26	6
17W	19.33	1.22	6	1.75	.40	6	6.97	.17	6	4.03	.09	6	2.93	.09	6	10.85	.20	6

DOSE : EXH-60M d

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	20.17	1.01	6	1.27 <sup>e</sup>	.12	6	6.40	.16	6	3.68	.11	6	2.72	.13	6	10.45	.11	6
13W	20.33	1.31	6	1.13 <sup>e</sup>	.10	6	6.45	.16	6	3.80 <sup>e</sup>	.07	6	2.65	.12	6	10.15 <sup>e</sup>	.10	6
17W	20.67	1.45	6	1.68	.23	6	6.88	.17	6	3.95	.07	6	2.93	.12	6	10.87	.03	6

## LEGEND:

- a32 day (6 week) exposure period.  
b65 day (13 week) exposure period.  
c30 day (4 week) post-exposure period.  
dDaily exposure period in minutes.  
eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P < 0.05$

\*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-9. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W a	3.97	.50	6	121.00	6.05	6	20.67	3.91	6	252.83	10.76	6	142.67	12.66	6	123.00	14.39	6		
13W b	4.55	.16	6	118.00	13.39	6	55.83	17.97	6	233.50	12.92	6	158.33	7.25	6	127.17	7.56	6		
17W c	4.55	.28	6	99.83	6.20	6	49.33	10.49	6	303.17	7.30	6	218.00	14.20	6	200.83	21.10	6		

DOSE : EXH-15M d

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	4.58	.31	5	115.40	6.40	5	23.60	5.33	5	252.80	17.76	5	128.60	11.63	5	98.40	11.55	5		
13W	4.07	.29	6	115.00	9.85	6	58.67	15.60	6	252.50	15.78	6	228.00	39.58	6	185.50	40.22	6		
17W	3.73	.30	6	99.00	4.12	6	44.33	11.45	6	274.67	12.51	6	183.50	26.06	6	179.33	30.81	6		

DOSE : EXH-60M d

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	4.02	.18	6	106.50	4.82	6	12.17	4.60	6	246.00	25.21	6	116.67	11.78	6	104.67	19.40	6		
13W	3.58	.20	5	98.33	6.03	6	38.50	11.52	6	246.67	12.82	6	153.50	10.73	6	148.17	14.78	6		
17W	3.97	.15	6	92.67	4.88	6	39.17	11.08	6	314.17	14.99	6	189.67	14.92	6	188.50	15.93	6		

## LEGEND:

- a32 day (6 week) exposure period.
- b65 day (13 week) exposure period.
- c30 day (4 week) post-exposure period.
- dDaily exposure period in minutes.
- eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P < 0.05$

\*Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m).

Table F-10. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust\*

SEX : MALE

DOSE : CTL

TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W <sup>a</sup>	200.17	4.88	6	19.00	.62	6	.67	.04	6	145.50	.39	6	4.47	.27	6	100.83	1.09	6
13W <sup>b</sup>	201.83	0.70	6	19.33	.73	6	.73	.04	6	144.50	1.05	6	4.78	.19	6	99.17	.72	6
17W <sup>c</sup>	203.67	8.17	6	20.33	.73	6	.67	.03	6	143.83	.72	6	4.65	.41	6	98.17	.98	6

DOSE : 2MGL-15W<sup>d</sup>

TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	188.50	10.87	6	17.50	.39	6	.68	.05	6	145.50	.57	6	4.38	.27	6	102.83	.98	6
13W	187.67	8.25	6	20.67	.90	6	.65	.07	6	145.67	.87	6	4.88	.31	6	100.33	.38	6
17W	222.17	14.97	6	20.83	.55	6	.68	.03	6	143.83	.86	6	4.75	.37	6	98.67	.51	6

DOSE : 2MGL-60W<sup>d</sup>

TIME	GLS			BUN			CRT			NA			K			CL		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	192.40	4.32	5	18.00	1.10	5	.52	.07	5	147.40 <sup>e</sup>	.36	5	4.38	.29	5	102.60	1.22	5
13W	234.00	6.46	6	17.67	.56	6	.62	.04	6	143.67	.45	6	4.80	.23	6	98.67	.51	6
17W	207.50	12.14	6	20.83	.55	6	.63	.03	6	144.67	.93	6	5.12	.26	6	98.67	.45	6

## LEGEND:

<sup>a</sup>32 day (6 week) exposure period.<sup>b</sup>65 day (13 week) exposure period.<sup>c</sup>30 day (4 week) post-exposure period.<sup>d</sup>Daily exposure period in minutes.

<sup>e</sup>Significant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P \leq 0.05$

\*Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg cu m).

Table F-11. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust\*

SEX : MALE

DOSE : CTL

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
6W	4.60	.23	6	87.67	5.14	6	42.00	6.65	6	244.67	37.44	6	106.17	9.15	6	105.33	10.32	6		
13W	4.88	.29	6	88.50	15.48	6	94.83	15.56	6	251.67	24.80	6	150.83	17.57	6	158.33	30.67	6		
17W	4.52	.20	6	67.00	3.80	6	105.00	26.55	5	325.20	22.92	5	120.20	12.38	5	120.80	14.15	5		

DOSE : 2MGL-15M d

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
06W	4.75	.15	6	76.00	5.96	6	32.50	10.61	6	238.33	18.88	6	105.17	17.46	6	79.17	7.50	6		
13W	5.48	.25	6	90.50	17.31	6	78.17	15.07	6	269.83	17.86	6	132.50	7.09	6	135.67	16.12	6		
17W	4.35	.17	6	64.67	2.81	6	94.83	16.47	6	309.17	13.08	6	122.00	12.88	6	115.33	11.74	6		

DOSE : 2MGL-60M d

TIME	PA			CHL			TRI			ALP			GOT			GPT				
	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N	MEAN	STD	ERR	N
6W	5.20	.13	5	87.60	5.63	5	19.40	5.07	5	238.20	11.74	5	125.00	27.16	5	118.60	19.01	5		
13W	5.05	.29	6	71.67	6.74	6	53.33	12.51	6	260.00	34.69	6	152.33	21.88	6	197.33	53.32	6		
17W	4.83	.36	6	65.17	3.05	6	82.83	9.41	6	298.83	32.54	6	153.07	23.54	6	140.50	21.36	6		

## LEGEND:

a32 day (6 week) exposure period.

b65 day (13 week) exposure period.

c30 day (4 week) post-exposure period.

dDaily exposure period in minutes.

eSignificant difference. Statistical evaluation included the Analysis of Variance and the Student's "t" test.  $P \leq 0.05$

\*Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m).

Table F-12. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W <sup>a</sup>	18.00	1.49	6	2.20	.28	6	6.75	.11	6	3.78	.08	6	2.97	.06	6	10.83	.17	6
13W <sup>b</sup>	17.83	1.16	6	2.10	.34	6	6.88	.08	6	4.20	.08	6	2.68	.08	6	10.75	.11	6
17W <sup>c</sup>	18.83	.98	6	1.59	.23	6	6.93	.19	6	4.00	.12	6	2.93	.08	6	10.90	.21	6

DOSE : 2MGL-15W<sup>d</sup>

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	19.17	1.83	6	1.47	.08	6	6.50	.16	6	3.77	.13	6	2.73	.10	6	10.52	.16	6
13W	18.83	1.36	6	1.67	.44	6	6.32	.14	6	3.82	.08	6	2.50	.08	6	10.33	.07	6
17W	19.00	.88	6	1.38	.11	6	6.80	.13	6	3.97	.08	6	2.83	.11	6	10.77	.09	6

DOSE : 2MGL-60W<sup>d</sup>

TIME	CD			UA			TP			AN			GLN			CA		
	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N	MEAN	STD ERR	N
06W	17.67	1.10	6	2.13	.33	6	6.48	.21	6	3.67	.17	6	2.82	.13	6	10.70	.23	6
13W	17.00	2.25	6	2.60	.43	6	6.68	.27	6	4.00	.20	6	2.68	.11	6	10.87	.22	6
17W	19.50	.96	6	1.90	.30	6	6.72	.14	6	3.88	.07	6	2.83	.09	6	10.83	.10	6

## LEGEND:

<sup>a</sup>32 day (6 week) exposure period.<sup>b</sup>65 day (13 week) exposure period.<sup>c</sup>30 day (4 week) post-exposure period.<sup>d</sup>Daily exposure period in minutes.

eSignificant difference. Statistical evaluation included the Analysis of Variance and the

Student's "t" test.  $P < 0.05$ \*Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg cu m).

Table F-13. Blood Chemical Effects on Fischer 344 Rats After Exposure by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust\*

SEX : FEMALE

DOSE : CTL

TIME	PA		CHL		TRI		ALP		GOT		GPT	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W a	3.97	.50	6.05	6	20.67	3.91	252.83	10.76	142.67	12.66	123.00	14.39
13W b	4.55	.16	119.00	13.39	55.83	17.97	233.50	12.92	158.33	7.25	127.17	7.56
17W c	4.55	.28	99.83	6.20	49.33	10.49	303.17	7.30	218.00	14.20	200.83	21.10

DOSE : 2MGL-15M d

TIME	PA		CHL		TRI		ALP		GOT		GPT	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W	4.65	.11	100.83	7.18	13.50	4.51	235.83	15.37	107.83	12.73	102.17	8.27
13W	4.17	.30	105.33	13.86	40.17	18.64	240.17	15.30	224.67	21.97	183.83	22.81
17W	4.02	.55	101.00	3.73	53.33	6.02	292.17	22.91	187.83	15.24	214.63	21.99

DOSE : 2MGL-60M d

TIME	PA		CHL		TRI		ALP		GOT		GPT	
	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR	MEAN	STD ERR
06W	4.62	.29	107.33	5.61	14.67	4.63	212.67	19.56	117.67	12.86	88.00	19.45
13W	4.95	.55	111.17	15.90	68.50	28.79	247.50	19.94	168.50	24.35	138.83	18.62
17W	4.07	.28	91.83	3.13	35.83	4.59	289.33	7.39	199.67	20.14	216.17	42.31

## LEGEND:

a32 day (6 week) exposure period.

b65 day (13 week) exposure period.

c30 day (4 week) post-exposure period.

dDaily exposure period in minutes.

eSignificant difference. Statistical evaluation included the Analysis of Variance and the

Student's "t" test.  $P < 0.05$ \*Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg cu m).

Blank



APPENDIX 6

THE RESULTS OF PULMONARY FUNCTION TESTS IN FISCHER 344 RATS  
FOLLOWING EXPOSURE TO DF2 (DIESEL FUEL) SMOKE AND/OR  
EXHAUST GENERATED BY THE M60A1 TANK

Blank

## SEX-Males

Table G-1. Results of Pulmonary Function Tests in Fischer 344 Rats Following Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

CONDITION AND TIME	# N	Estimated Pulmonary Resistance <sup>a</sup> (cm H <sub>2</sub> O/l/Sec)	# N	Rate (Breaths/ Minute) <sup>a</sup>	# N	Minute Volume <sup>a</sup> (ml)	# N	Tidal Volume <sup>a</sup> (ml)
1. 32-Day Exposure to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	5	80.5 ± 6.3	6	173.0 ± 9.7	3	232.1 ± 13.3	3	1.39 ± 0.07
15 Minute Exposure	6	84.7 ± 15.6	6	164.0 ± 19.9	4	247.1 ± 15.8	4	1.33 ± 0.10
60 Minute Exposure	5	74.9 ± 7.53	6	167.0 ± 11.4	4	211.2 ± 11.0	4	1.33 ± 0.14
2. 65-Day Exposure to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	6	74.81 ± 2.7	6	163.0 ± 5.4	6	278.1 ± 12.1	6	1.72 ± 0.09
15 Minute Exposure	6	81.80 ± 2.5	6	171.0 ± 11.9	6	270.9 ± 19.2	6	1.73 ± 0.09
60 Minute Exposure	6	74.80 ± 2.7	6	163.0 ± 5.4	6	278.1 ± 12.1	6	1.72 ± 0.09
3. 30 Days Post-Exposure After 65 Exposures to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	6	84.5 ± 3.9	6	151.0 ± 6.6	-	c	-	c
15 Minute Exposure	6	79.5 ± 0.10	6	173.0 ± 10.2	-	c	-	c
60 Minute Exposure	6	83.3 ± 7.3	6	143.0 ± 10.2	-	c	-	c

<sup>a</sup>Mean and standard error - statistical analysis is with Student's "t" test ( $p < 0.05$ )<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)<sup>c</sup>Animal values not evaluated.

## SEX-Females

Table G-2. Results of Pulmonary Function Tests in Fischer 344 Rats Following Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

CONDITION AND TIME	# N	Estimated Pulmonary Resistance <sup>a</sup> (cm H <sub>2</sub> O/l/Sec)	# N	Rate (Breaths/Minute)	# N	Minute Volume <sup>a</sup> (ml)	# N	Tidal Volume <sup>a</sup> (ml)
1. 32-Day Exposure to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	5	63.3 + 7.5	5	143.0 + 21.1	3	156.0 + 19.2	3	1.30 + 0.25
15 Minute Exposure	6	61.9 + 10.1	6	140.0 + 12.9	4	204.8 + 37.2	4	1.17 + 0.16
60 Minute Exposure	5	74.9 + 7.53	6	167.0 + 11.4	4	211.2 + 11.0	4	1.33 + 0.14
2. 65-Day Exposure to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	6	67.1 + 2.1	6	165.0 + 10.1	6	187.5 + 4.9	6	1.16 + 0.07
15 Minute Exposure	6	56.7 + 2.4	6	168.0 + 6.2	6	189.9 + 6.4	6	1.15 + 0.07
60 Minute Exposure	6	67.1 + 2.1	6	166.0 + 10.1	6	187.5 + 4.9	6	1.16 + 0.07
3. 30 Days Post-Exposure After 65 Exposures to DF2 Smoke/Exhaust <sup>b</sup>								
Controls	6	64.4 + 3.8	6	165.0 + 10.0	-	c	-	c
15 Minute Exposure	6	67.0 + 2.1	6	172.0 + 5.3	-	c	-	c
60 Minute Exposure	6	83.3 + 7.3	6	143.0 + 10.2	-	c	-	c

<sup>a</sup>Mean and standard error - statistical analysis is with Student's "t" test (p<0.05)<sup>b</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust = 2.340 + 0.450 mg/l (2,340 + 450 mg/cu m)<sup>c</sup>Animal values not evaluated.

## SEX-Males

Table G-3. Results of Pulmonary Function Tests in Fischer 344 Rats Following Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

CONDITION AND TIME	# N	Estimated Pulmonary Resistance <sup>a</sup> (cm H <sub>2</sub> O/l/Sec)	# N	Rate (Breaths/Minute)	# N	Minute Volume <sup>a</sup> (ml)	# N	Tidal Volume <sup>a</sup> (ml)
1. 32-Day Exposure to DF2 Exhaust <sup>b</sup>								
Controls	5	80.5 ± 6.3	6	173.0 ± 9.7	3	232.1 ± 13.3	3	1.39 ± 0.07
15 Minute Exposure	5	70.6 ± 3.2	6	168.0 ± 9.3	3	222.3 ± 15.9	3	1.25 ± 0.16
60 Minute Exposure	6	76.6 ± 8.4	6	163.0 ± 17.3	4	167.0 ± 39.2	4	1.00 ± 0.17
2. 65-Day Exposure to DF2 Exhaust <sup>b</sup>								
Controls	6	74.8 ± 2.70	6	163.0 ± 5.40	6	278.10 ± 12.10	6	1.72 ± 0.09
15 Minute Exposure	6	65.7 ± 4.90	6	164.5 ± 12.20	6	241.10 ± 19.70	6	1.48 ± 0.09
60 Minute Exposure	6	71.3 ± 10.40	6	181.0 ± 14.80	6	258.80 ± 12.10	6	1.47 ± 0.12
3. 30 Days Post-Exposure After 65 Exposures to DF2 Exhaust <sup>b</sup>								
Controls	6	84.5 ± 3.9	6	151.0 ± 6.6	-	c	-	c
15 Minute Exposure	6	85.6 ± 3.8	6	148.0 ± 7.9	-	c	-	c
60 Minute Exposure	6	85.2 ± 4.6	6	159.0 ± 5.9	-	c	-	c

<sup>a</sup>Mean and standard error - statistical analysis is with Student's "t" test ( $p < 0.05$ )<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)<sup>c</sup>Animal values not evaluated.

## SEX-Females

Table G-4. Results of Pulmonary Function Tests in Fischer 344 Rats Following Exposure to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

CONDITION AND TIME	# N	Estimated Pulmonary Resistance <sup>a</sup> (cm H <sub>2</sub> O/l/Sec)	# N	Rate (Breaths/Minute) <sup>a</sup>	# N	Minute Volume (ml) <sup>a</sup>	# N	Tidal Volume (ml) <sup>a</sup>
1. 32-Day Exposure to DF2 Exhaust <sup>b</sup>								
Controls	5	63.3 ± 7.5	5	143.0 ± 21.1	3	156.9 ± 19.2	3	1.30 ± 0.25
15 Minute Exposure	6	56.8 ± 4.6	6	175.0 ± 9.1	4	165.3 ± 3.7	4	0.98 ± 0.11
60 Minute Exposure	6	53.3 ± 3.29	6	164.0 ± 17.1	4	168.8 ± 13.3	4	0.93 ± 0.09
2. 65-Day Exposure to DF2 Exhaust <sup>b</sup>								
Controls	6	67.10 ± 2.10	6	165.0 ± 10.1	6	187.50 ± 4.90	6	1.16 ± 0.07
15 Minute Exposure	6	71.80 ± 3.70	6	169.0 ± 5.90	6	185.30 ± 12.50	6	1.09 ± 0.08
60 Minute Exposure	6	70.80 ± 4.60	6	157.0 ± 13.80	6	209.40 ± 3.40	6	1.33 ± 0.12
3. 30 Days Post-Exposure After 65 Exposures to DF2 Exhaust <sup>b</sup>								
Controls	5	64.4 ± 2.5	5	177.0 ± 6.9	-	c	-	c
15 Minute Exposure	7	70.9 ± 2.5	7	165.0 ± 8.6	-	c	-	c
60 Minute Exposure	6	64.4 ± 3.8	6	165.0 ± 10.0	-	c	-	c

<sup>a</sup>Mean and standard error - statistical analysis is with Student's "t" test ( $p < 0.05$ )<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)<sup>c</sup>Animal values not evaluated.

## APPENDIX H

THE PHYSIOLOGICAL MEASUREMENTS OF FISCHER 344 RATS DURING AND  
AFTER EXPOSURE BY THE AIRBORNE ROUTE TO DF2 (DIESEL FUEL)  
SMOKE AND/OR EXHAUST GENERATED BY THE M60A1 TANK

Blank



Table H-1. Physiological Measurements on Fischer 344 Rats Exposed by the Airborne Route for 32 Daily Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Measurement	Units	EXPOSURE CONDITION										ANOVA		
		Control					15 Min Exhaust					Significance of F Ratio		
		All	M	F	All	M	F	All	M	F	All	M	F	Dose
Weight	Grams	273+	348+	199+	289+	347+	190+	271+	344+	198+	288+	194+	341+	
Temp	°C	84	43	17	88	44	15	83	40	24	83	22	39	.05
Rectal		7	7	7	1.3	1.7	0.5	1.1	0.3	1.3	1.2	0.9	1.5	.5
Tidal	ml	2.04+	2.24+	1.80+	1.78+	1.91+	1.66+	1.90+	2.14+	1.66+	1.79+	1.91+	1.67+	.05
Volume		0.52	0.52	0.47	0.56	0.60	0.34	0.58	0.49	0.58	0.41	0.44	0.37	.05
Minute	ml	276+	310+	235+	228+	244+	212+	250+	300+	199+	227+	246+	208+	.05
Volume		85	93	59	59	55	63	74	65	43	34	28	30	
Breath.	B/min	135+	137+	131+	130+	130+	130+	130+	141+	120+	130+	132+	128+	
Freq.	min	13	13	13	15	15	17	23	29	9	19	2	20	
Tidal Vol.	ml	2.68+	2.85+	2.48+	2.57+	2.68+	2.47+	2.64+	2.97+	2.30+	2.53+	2.61+	2.45+	.05
6% CO2		0.59	0.53	0.65	0.61	0.61	0.65	0.50	0.40	0.34	0.45	0.40	0.53	
Min. Vol.	ml	424+	456+	385+	415+	413+	417+	431+	513+	349+	409+	426+	374+	.05
6% CO2		78	69	76	117	92	148	100	53	54	81	52	94	
Breath f	B/min	160+	162+	158+	160+	154+	166+	164+	176+	152+	160+	165+	154+	
6% CO2		16	17	16	18	13	21	28	34	12	25	26	26	
B.P.	mm	114+	115+	114+	116.3+	124+	109+	118+	128+	108+	116+	118+	113+	
Systolic	Hg	7	9	7	11	7	8	15	14	6	5	6	4	
H.R. from	B/min	472+	477+	468+	423+	438+	408+	453+	481+	424+	452+	461+	444+	
B.P.		26	34	18	34	37	27	59	52	52	40	41	41	
H.R. from	B/min	473+	457+	492+	474+	469+	478+	464+	455+	472+	455+	498+	485+	.5
ECG		46	61	20	59	80	38	43	55	30	29	34	16	
P amp	mv	0.34+	0.36+	0.32+	0.49+	0.42+	0.58+	0.48+	0.43+	0.53+	0.37+	0.44+	0.32+	
EKG		0.12	0.01	0.13	0.14	0.13	0.10	0.26	0.30	0.21	0.24	0.16	0.19	
R amp	mv	0.329+	0.322+	0.336+	0.338+	0.324+	0.352+	0.312+	0.293+	0.330+	0.339+	0.363+	0.388+	
EKG		0.94	1.13	0.82	0.68	0.54	0.83	1.22	0.94	1.53	1.00	1.02	0.83	
T amp	mv	0.40+	0.22+	0.58+	0.15+	0.98+	0.12+	1.03+	0.72+	1.33+	0.80+	0.67+	0.68+	.5
EKG		0.44	0.35	0.48	0.18	0.23	0.11	0.73	0.49	0.84	0.48	0.36	0.41	
P dur	sec	0.016+	0.016+	0.016+	0.016+	0.016+	0.016+	0.016+	0.015+	0.016+	0.017+	0.016+	0.017+	
EKG		0.000	0.000	0.000	0.001	0.002	0.000	0.001	0.001	0.001	0.001	0.001	0.001	.05
PR int	sec	0.040+	0.040+	0.040+	0.039+	0.039+	0.038+	0.040+	0.040+	0.039+	0.040+	0.040+	0.040+	
EKG		0.002	0.003	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.001	0.001	
QRS int	sec	0.018+	0.018+	0.018+	0.018+	0.017+	0.016+	0.017+	0.018+	0.018+	0.017+	0.017+	0.019+	
EKG		0.002	0.002	0.001	0.001	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.002	
QT int	sec	0.063+	0.063+	0.063+	0.061+	0.062+	0.060+	0.064+	0.063+	0.064+	0.063+	0.064+	0.062+	
Axis	o	0.004	0.005	0.003	0.008	0.006	0.011	0.002	0.002	0.002	0.004	0.004	0.005	
Readmill	min	35+	37+	33+	40+	39+	42+	36+	36+	34+	34+	28+	39+	
Run		14	11	17	15	15	17	16	17	16	15	17	20	
		9.5+	8.2+	11.0+	11.0+	9.1+	12.9+	11.1+	8.2+	13.5+	9.4+	9.8+	12.7+	.05
		3.7	3.5	3.8	4.5	4.3	4.0	4.1	8.2	2.0	5.0	4.8	3.7	

a Average total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) smoke/exhaust = 2.340 = 0.450 mg/l (2,340 + 450 mg/cu m)  
b Average total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) exhaust = 0.006 + 0.006 mg/l (6.0 + 6.0 mg/cu m)  
c ANOVA = Analysis of Variance.

Table H-2. Physiological Measurements on Fischer 344 Rats Exposed by the Airborne Route for 65 Daily Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke and/or Exhaust

Measurement		Units	EXPOSURE CONDITION												ANOVA				
			Control			15 Min Exhaust			15 Min Smoke+Exh			60 Min Exhaust			60 Min Smoke+Exh			Significance of F Ratio	
			All	M	F	All	M	F	All	M	F	All	M	F	All	M	F	Male	Female
Weight	Grams	300+ 96	388+ 40	211+ 11	287+ 93	366+ 63	208+ 6	291+ 89	373+ 33	209+ 11	308+ 103	292+ 95	378+ 45	206+ 12			.05		
Temp	°F	101.5+ 0.8	101.4+ 0.6	101.7+ 0.7	101.1+ 0.8	100.8+ 0.7	101.4+ 0.8	101.2+ 1.2	100.7+ 1.3	101.8+ 0.8	101.7+ 0.9	102.0+ 0.5	101.9+ 0.5	102.1+ 0.6			.05		
Rectal																			
Tidal	ml	1.66+ 31	1.84+ 29	1.48+ 23	1.52+ 22	1.64+ 26	1.40+ 05	1.65+ 31	1.90+ 21	1.40+ 14	1.87+ 47	1.91+ 45	2.25+ 30	1.57+ 26			.05		
Volume	ml	217+ 61	252+ 61	183+ 39	181+ 25	202+ 10	161+ 15	207+ 49	242+ 47	172+ 13	219+ 58	230+ 52	263+ 38	191+ 45			.05		
Minute	ml																		
Volume	B/	130+ 17	136+ 19	123+ 14	119+ 16	125+ 18	115+ 13	126+ 18	127+ 14	125+ 22	116+ 14	120+ 13	117+ 10	123+ 15					
Breath.	min																		
Freq.	min	2.31+ 33	2.52+ 24	2.09+ 27	2.27+ 38	2.36+ 41	2.17+ 36	2.18+ 41	2.48+ 28	1.89+ 30	2.38+ 44	2.28+ 68	2.81+ 44	1.75+ 38			.05		
Tidal Vol.	ml																		
6% CO2	ml	339+ 70	369+ 85	310+ 39	314+ 77	314+ 71	314+ 90	320+ 80	353+ 80	287+ 71	307+ 55	310+ 125	385+ 102	385+ 103			.05		
Min. Vol.	ml																		
6% CO2	B/	147+ 21	149+ 5	146+ 31	138+ 22	144+ 14	132+ 28	146+ 21	142+ 21	150+ 21	130+ 24	133+ 31	129+ 38	136+ 24					
Breath f	min																		
6% CO2	mm	114+ 6	115+ 7	113+ 7	120+ 14	129+ 15	111+ 7	115+ 10	116+ 13	114+ 7	113+ 11	120+ 9	123+ 8	117+ 10			.5		
B.P.	Hg																		
Systolic	B/	451+ 31	457+ 38	444+ 23	449+ 55	463+ 53	436+ 59	472+ 27	471+ 22	472+ 35	451+ 16	462+ 61	478+ 58	447+ 54					
H.R. from	min																		
6% CO2	B/	472+ 41	479+ 30	465+ 51	462+ 30	466+ 36	459+ 25	455+ 50	438+ 73	472+ 43	436+ 23	446+ 42	430+ 35	462+ 44					
H.R. from	min																		
EKG	mv	0.28+ 0.021	0.22+ 0.021	0.33+ 0.020	0.43+ 0.018	0.37+ 0.014	0.50+ 0.020	0.41+ 0.018	0.33+ 0.016	0.48+ 0.017	0.35+ 0.015	0.43+ 0.013	0.38+ 0.013	0.410+ 0.012			.5		
P amp	mv																		
EKG	mv	0.37+ 0.103	0.32+ 0.099	0.48+ 0.090	0.42+ 0.131	0.36+ 0.077	0.48+ 0.154	0.38+ 0.101	0.39+ 0.133	0.37+ 0.067	0.37+ 0.103	0.39+ 0.139	0.37+ 0.097	0.410+ 0.179					
R amp	mv																		
EKG	mv	0.08+ 0.04	0.08+ 0.03	0.08+ 0.06	0.12+ 0.08	0.10+ 0.09	0.13+ 0.06	0.10+ 0.05	0.09+ 0.05	0.12+ 0.05	0.10+ 0.04	0.11+ 0.05	0.06+ 0.03	0.13+ 0.04			.5		
I amp	mv																		
EKG	mv	0.017+ 0.001	0.016+ 0.001	0.017+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.016+ 0.001	0.017+ 0.001	0.016+ 0.000					
P dur	sec																		
EKG	sec	0.039+ 0.001	0.038+ 0.001	0.039+ 0.001	0.038+ 0.001	0.040+ 0.002	0.036+ 0.002	0.040+ 0.002	0.039+ 0.002	0.039+ 0.002	0.040+ 0.001	0.040+ 0.002	0.041+ 0.002	0.039+ 0.001					
PR int	sec																		
EKG	sec	0.018+ 0.001	0.017+ 0.001	0.018+ 0.002	0.017+ 0.002	0.017+ 0.002	0.018+ 0.002	0.017+ 0.001	0.017+ 0.001	0.018+ 0.001	0.017+ 0.002	0.017+ 0.002	0.018+ 0.001	0.018+ 0.002					
QRS int	sec																		
EKG	sec	0.061+ 0.003	0.062+ 0.003	0.061+ 0.002	0.063+ 0.003	0.063+ 0.004	0.063+ 0.003	0.063+ 0.003	0.063+ 0.002	0.062+ 0.003	0.061+ 0.002	0.063+ 0.002	0.063+ 0.004	0.063+ 0.005					
QT int	sec																		
EKG	sec	0.040+ 0.022	0.044+ 0.026	0.046+ 0.026	0.046+ 0.027	0.046+ 0.022	0.047+ 0.030	0.047+ 0.019	0.041+ 0.022	0.041+ 0.022	0.041+ 0.019	0.041+ 0.019	0.041+ 0.019	0.041+ 0.019					
Axis	°																		
Treadmill	min	7.4+ 3.5	6.4+ 4.3	8.5+ 2.2	7.1+ 3.6	6.2+ 2.4	8.1+ 4.6	9.2+ 4.9	5.2+ 3.0	9.2+ 4.9	4.8+ 2.4	5.3+ 1.7	3.1+ 1.4	5.1+ 2.5			.5		

aAverage total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) smoke/exhaust =

2.340 + 0.450 mg/l (2,340 + 0.45 mg/cu m)

bAverage total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) exhaust =

0.006 + 0.006 mg/l (6.0 + 6.0 mg/cu m)

CANOVA = Analysis of Variance

Table H-3. Physiological Measurements on Fischer 344 Rats, Four Weeks (30 days) After 65 Daily Airborne Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoked and/or Exhaust<sup>b</sup>

Measurement	Units	EXPOSURE CONDITION												ANOVA							
		Control						15 Min Exhaust						60 Min Exhaust						Significance of F Ratio	
		All	M	F	All	M	F	All	M	F	All	M	F	All	M	F	Dose	Male	Female		
Weight	Grams	323+	429+	217+	290+	421+	216+	322+	421+	223+	312+	430+	213+	315+	402+	228+					
		114	36	16	107	46	18	107	36	12	118	48	10	94	27	19			.05		
Temp	°F	100.5+	100.1+	101.0+	100.3+	100.0+	100.5+	100.5+	100.3+	100.6+	100.5+	100.1+	100.8+	100.6+	100.5+	100.6+					
		0.8	0.9	0.5	0.8	1.0	0.8	0.8	1.0	0.6	1.0	1.3	0.4	0.9	1.2	0.7			.05		
Rectal		1.96+	2.39+	1.53+	1.67+	2.02+	1.47+	1.93+	2.36+	1.49+	1.78+	2.05+	1.55+	1.86+	2.16+	1.57+					
		.59	.52	.20	.34	.10	.24	.53	.38	.12	.33	.20	.24	.54	.57	.31			.05		
Tidal	ml	192+	246+	138+	162+	218+	130+	188+	238+	137+	168+	216+	128+	191+	228+	152+					
Minute	ml	92	107	16	56	54	22	65	53	21	50	23	16	81	97	38			.05		
Volume	B/	95+	99+	91+	96+	108+	89+	96+	101+	92+	94+	106+	84+	101+	103+	98+					
Breath.	min	16	20	9	16	22	7	15	19	10	19	12	19	17	19	17			.05		
Free.																					
Tidal Vol.	ml	2.92+	3.55+	2.30+	2.72+	3.23+	2.43+	2.85+	3.46+	2.24+	2.91+	3.61+	2.33+	3.02+	3.51+	2.54+			.05		
6% CO2		.71	.32	.25	.41	.09	.13	.74	.49	.29	.72	.34	.22	.57	.32	.21					
Min. Vol.	ml	433+	520+	347+	413+	497+	365+	422+	516+	327+	466+	599+	356+	486+	587+	385+			.05		
6% CO2		103	59	44	85	71	45	116	73	52	117	58	53	117	67	34					
Breath f	B/	148+	146+	149+	152+	154+	151+	148+	150+	146+	159+	166+	153+	160+	167+	152+					
6% CO2	min	13	10	16	19	18	21	13	13	14	15	7	18	12	8	10					
B.P.	mm	117+	122+	113+	116+	118+	114+	116+	120+	111+	114+	121+	108+	117.2+	122+	112+			.05		
Systolic	Hg	8	8	4	5	7	3	7	7	4	11	11	4	7	7	4					
H.R. from	B/	447+	467+	428+	433+	444+	426+	468+	469+	468+	435+	449+	432+	458+	454+	462+					
B.P.	min	45	43	41	36	43	32	34	33	35	35	46	20	29	24	35					
H.R. from	B/	453+	442+	464+	439+	419+	451+	460+	430+	490+	465+	439+	488+	474+	458+	491+					
EKG	min	39	37	41	35	15	39	49	26	50	39	50	37	40	40	35			.05		
P amp	mv	.049+	.052+	.047+	.043+	.040+	.044+	.038+	.043+	.032+	.034+	.036+	.032+	.043+	.040+	.044+					
EKG	mv	.023	.026	.021	.017	.014	.019	.013	.008	.015	.010	.013	.008	.017	.014	.020					
R amp	mv	.384+	.305+	.463+	.379+	.395+	.370+	.423+	.392+	.455+	.406+	.380+	.427+	.379+	.395+	.370+					
EKG	mv	.143	.088	.149	.100	.076	.117	.127	.066	.170	.139	.142	.145	.100	.076	.117					
I amp	mv	.107+	.095+	.118+	.081+	.088+	.077+	.094+	.115+	.077+	.073+	.074	.072+	.081+	.088+	.077+					
EKG	mv	.031	.024	.035	.043	.021	.053	.039	.026	.039	.030	.043	.018	.043	.021	.053					
P dur	sec	.016+	.016+	.016+	.016+	.017+	.015+	.016+	.017+	.016+	.017+	.016+	.017+	.016+	.017+	.015+					
EKG	sec	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.002	.001	.001	.001	.001					
PR int	sec	.040+	.039+	.040+	.040+	.041+	.039+	.040+	.042+	.039+	.040+	.042	.039+	.040+	.040+	.039+			.5		
EKG	sec	.001	.002	.001	.001	.001	.001	.002	.002	.002	.002	.003	.004	.001	.001	.001					
QRS int	sec	.018+	.018+	.017+	.017+	.018+	.017+	.018+	.017+	.018+	.018+	.018+	.019+	.017+	.018+	.017+					
EKG	sec	.001	.002	.001	.001	.001	.001	.001	.001	.001	.002	.002	.002	.001	.001	.001					
QT int	sec	.059+	.058+	.060+	.060+	.060+	.060+	.059+	.059+	.059+	.060+	.059	.061+	.060+	.060+	.060+					
EKG	sec	.002	.002	.002	.001	.002	.001	.002	.003	.002	.005	.004	.005	.001	.001	.001					
QT int	sec	.30+	.26+	.33+	.33+	.34+	.33+	.37+	.24+	.49+	.30+	.29	.32+	.34+	.38+	.29+					
EKG	sec	.19	.14	.23	.11	.10	.13	.27	.20	.26	.20	7	7	15	13	17					
Axis	°																				
Treadmill	min																				
Run																					

a Average total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) smoke/exhaust =  $2.340 \pm 0.450 \text{ mg/l}$  ( $2.340 \pm 0.45 \text{ mg/cu m}$ )

b Average total hydrocarbon concentration of M60A1 tank-generated DF2 (Diesel Fuel) exhaust =  $0.006 \pm 0.006 \text{ mg/l}$  ( $6.0 \pm 6.0 \text{ mg/cu m}$ )

CANOVA = Analysis of Variance

Blank

APPENDIX I

STATISTICAL MEANS AND STANDARD ERRORS FROM TESTS USED TO EVALUATE  
THE BEHAVIORAL PERFORMANCE OF FISCHER 344 RATS DURING AND  
AFTER AIRBORNE EXPOSURE TO DF2 (DIESEL FUEL) SMOKE  
AND/OR EXHAUST GENERATED BY THE M60A1 TANK

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Table I-1. Statistical Means and Standard Errors From Tests Used to Evaluate the Behavioral Performance of Fischer 344 Rats Exposed for 32 Daily Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

Sex & Treatment Group*	BODY WEIGHT		PASSIVE AVOIDANCE TEST (PAT)			SPONTANEOUS ACTIVITY TEST (SAT)			
	Grams: M (+ SE)	# Shocks M (+ SE)	Time in Shock (Min) M (+ SE)	# PAR's M (+ SE)	Gross (G) Move- ment M (+ SE)	Fine (F) Move- ment M (+ SE)	Ratio F/G M (+ SE)		
M-C	326 (+16)	17.8 (+5.0)	2.20 (+0.58)	9.5 (+2.0)	678 (+124)	1432 (+170)	2.22 (+0.20)		
F-C *	187 (+ 8)	5.0 (+2.4)	0.68 (+0.30)	6.6 (+1.5)	645 (+ 62)	1019 (+ 85)	1.65 (+0.22)		
M-E-15	321 (+20)	13.8 (+2.5)	1.67 (+0.23)	5.3 (+1.1)	750 (+101)	1138 (+ 99)	1.67 (+0.27)		
F-E-15	181 (+16)	8.2 (+2.4)	0.88 (+0.26)	4.0 (+0.5)	782 (+ 68)	1060 (+140)	1.39 (+0.17)		
M-E-60	334 (+22)	14.0 (+2.8)	1.75 (+0.26)	8.7 (+1.6)	649 (+161)	809 (+ 86)	1.51 (+0.23)		
F-E-60	183 (+ 6)	12.7 (+3.2)	1.63 (+0.39)	9.5 (+2.4)	625 (+ 50)	1141 (+ 76)	1.88 (+0.17)		
M-S-15*	324 (+18)	16.2 (+5.9)	2.37 (+0.68)	10.2 (+2.1)	603 (+ 72)	995 (+160)	1.62 (+0.11)		
F-S-15	188 (+ 8)	9.7 (+2.5)	1.26 (+0.26)	9.7 (+2.9)	766 (+112)	1204 (+101)	1.64 (+0.09)		
M-S-60	320 (+14)	12.8 (+3.5)	1.54 (+0.37)	7.7 (+2.4)	656 (+ 45)	1097 (+110)	1.70 (+0.16)		
F-S-60	184 (+ 7)	8.3 (+2.4)	1.06 (+0.34)	5.3 (+1.1)	657 (+ 50)	1196 (+142)	1.86 (+0.25)		

LEGEND:

M = male rats

F = female rats

C = controls (60 minute air exposures)

S = DF2 smoke/exhaust (15 or 60 minute exposures)

E = DF2 exhaust (15 or 60 minute exposures)

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\*N=5, for all other conditions N=6.

Table I-2. Statistical Means and Standard Errors From Tests Used to Evaluate the Behavioral Performance of Fischer 344 Rats Exposed for 65 Daily Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

Sex & Treatment Group*	BODY WEIGHT		PASSIVE AVOIDANCE TEST (PAT)			SPONTANEOUS ACTIVITY TEST (SAT)				
	Grams: M (+ SE)	# Shocks M (+ SE)	Time in Shock (Min) M (+ SE)	# PAR's M (+ SE)	Gross (G) Move- ment M (+ SE)	Fine (F) Move- ment M (+ SE)	Ratio F/G M (+ SE)			
M-C	359 (+14)	11.5 (+9.0)	1.19 (+0.93)	6.3 (+5.8)	488 (+113)	1017 (+464)	2.05 (+0.35)			
F-C	197 (+4)	4.7 (+1.7)	0.81 (+0.21)	10.0 (+5.9)	494 (+206)	949 (+340)	2.04 (+0.28)			
M-E-15	363 (+9)	9.0 (+9.7)	0.73 (+0.72)	5.3 (+4.5)	413 (+181)	715 (+177)	2.08 (+0.53)			
F-E-15	195 (+2)	4.8 (+4.6)	0.56 (+0.46)	4.8 (+3.1)	513 (+235)	952 (+459)	1.78 (+0.18)			
M-E-60	376 (+13)	12.2 (+15.3)	1.19 (+1.36)	5.3 (+3.9)	490 (+158)	786 (+119)	1.81 (+0.30)			
F-E-60	201 (+9)	6.7 (+4.4)	0.8 (+0.62)	7.0 (+7.2)	637 (+248)	1077 (+232)	1.84 (+0.23)			
M-S-15	347 (+11)	9.0 (+5.0)	1.15 (+0.54)	6.2 (+1.5)	553 (+117)	987 (+271)	1.86 (+0.29)			
F-S-15	197 (+4)	6.7 (+2.9)	0.76 (+0.35)	4.5 (+3.0)	578 (+161)	1175 (+389)	2.12 (+0.38)			
M-S-60	360 (+15)	12.2 (+6.9)	1.4 (+0.77)	5.3 (+5.4)	511 (+177)	899 (+331)	1.89 (+0.31)			
F-S-60	194 (+4)	6.2 (+3.7)	0.74 (+0.43)	6.0 (+3.6)	550 (+75)	1193 (+198)	2.20 (+0.17)			

LEGEND:

M = male rats

F = female rats

C = controls (60 minute air exposures)

S = DF2 smoke/exhaust (15 or 60 minute exposures)

E = DF2 exhaust (15 or 60 minute exposures)

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\*N= 6



Table I-3. Statistical Means and Standard Errors From Tests Used to Evaluate the Behavioral Performance of Fischer 344 Rats, Thirty Days After Sixty-Five Daily Exposures to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup>

Sex & Treatment Group*	BODY WEIGHT			PASSIVE AVOIDANCE TEST (PAT)			SPONTANEOUS ACTIVITY TEST (SAT)			
	Grams: M (+ SE)	# Shocks M (+ SE)	Time in Shock (Min) M (+ SE)	# PAR's M (+ SE)	Gross (G) Move- ment M (+ SE)	Fine (F) Move- ment M (+ SE)	Ratio F/G M (+ SE)			
M-C	398 (+14)	9.2 (+1.9)	1.18 (+0.25)	6.5 (+0.9)	636 (+84)	1091 (+105)	1.86 (+0.25)			
F-C	204 (+ 6)	6.0 (+2.2)	0.71 (+0.26)	5.5 (+1.9)	582 (+125)	1169 (+229)	2.34 (+0.48)			
M-E-15 *	385 (+20)	9.8 (+4.1)	1.03 (+0.47)	4.0 (+1.7)	503 (+ 62)	1408 (+269)	3.08 (+0.83)			
F-E-15	203 (+ 9)	2.8 (+0.5)	0.32 (+0.08)	2.5 (+1.1)	408 (+ 50)	828 (+ 74)	2.18 (+0.30)			
M-E-60 **	400 (+18)	7.8 (+1.6)	0.84 (+0.18)	2.8 (+0.6)	675 (+ 75)	1032 (+156)	1.63 (+0.32)			
F-E-60	201 (+ 3)	9.3 (+2.4)	1.26 (+0.24)	9.3 (+1.5)	493 (+ 43)	1169 (+154)	2.34 (+0.23)			
M-S-15	401 (+ 6)	9.3 (+3.7)	1.07 (+0.39)	6.3 (+1.7)	633 (+ 29)	897 (+110)	1.40 (+0.14)			
F-S-15	211 (+ 6)	8.3 (+3.1)	1.09 (+0.28)	5.5 (+1.9)	603 (+ 85)	1408 (+228)	2.60 (+0.54)			
M-S-60	381 (+11)	5.2 (+1.0)	0.65 (+0.14)	3.9 (+1.1)	631 (+ 87)	1199 (+173)	1.90 (+0.18)			
F-S-60	214 (+ 7)	3.3 (+1.1)	0.50 (+0.15)	6.0 (+2.0)	472 (+ 98)	1140 (+ 532)	2.54 (+0.32)			

## LEGEND:

M = male rats

F = female rats

C = controls (60 minute air exposures)

S = DF2 smoke/exhaust (15 or 60 minute exposures)

E = DF2 exhaust (15 or 60 minute exposures)

\*N = 4

\*\*N = 5

All other conditions, N = 6

<sup>a</sup>Average total hydrocarbon concentration of DF2 smoke/exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup>Average total hydrocarbon concentration of DF2 exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

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APPENDIX J

TOXIC AND/OR MUTAGENIC EFFECTS IN DROSOPHILA MELANOGASTER (FRUIT FLY)  
AFTER ORAL EXPOSURE TO DF2 DIESEL FUEL OR INHALATION EXPOSURE TO  
DF2 (DIESEL FUEL) SMOKE/EXHAUST GENERATED BY THE M60A1 TANK

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Table J-1. Toxic Effects in *Drosophila melanogaster* After Oral Exposure for 72-Hours to DF2 Diesel Fuel

Test substance	Concentration of DF2 Diesel Fuel (%)	No. of <i>Drosophila melanogaster</i> exposed	% Mortality
DF2 (Diesel Fuel)	10.0 <sup>a</sup>	110	77
	1.0 <sup>a</sup>	110	4
	0.1 <sup>a</sup>	110	5
Distilled water <sup>b,*</sup>		345	6
Ethanol-10% <sup>c,*</sup>		110	0
Methymethane sulfonated <sup>d,**</sup> - 0.5 $\mu$ m		225	66

LEGEND:

\*Negative control

\*\*Positive control

<sup>a</sup>percent of DF2 (Diesel Fuel) added to 1.5 ml of ethanol diluted with 15 ml of distilled water and added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic vials.

<sup>b</sup>15.0 ml of distilled water added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

<sup>c</sup>1.5 ml of ethanol diluted to 15.0 ml with distilled water and added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

<sup>d</sup>0.5  $\mu$ m of methymethane sulfonate mixed with 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

Table J-2. Toxic Effects in *Drosophila* Melanogaster After Exposure by the Inhalation Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust

Exposure substance	Average total hydrocarbon concentration in exposure chamber (mg/l)	Duration of exposure (min)	No. of daily exposures	Exposure container description	No. of <i>Drosophila</i> melanogaster exposed	Mortality* (%)
M60A1 Tank-Generated DF2	2,340 $\pm$ 0.450	60	5	Glass tube, (35 mm dia x 200 mm long)	200	8
Diesel Fuel	(2,340 $\pm$ 450 mg/cu m)			33 mm dia screen capped one end		
Smoke/Exhaust		60	4	Wire screen cylinder (22 mm dia x 160 mm long)	100	18

\*Organisms were observed for mortality at 24 hours after the last exposure.

Table J-3. Mutagenic Effects in *Drosophila Melanogaster* After Oral Exposure for 72-Hours to DF2 Diesel Fuel

Test substance	Concentration of DF2 Diesel Fuel (%)	X-Chromosomes tested (no.)	Mortality (no.)	Mortality (%)
DF2 (Diesel Fuel)	10.0 <sup>a</sup>	105	0	0
	1.0 <sup>a</sup>	106	0	0
	0.1 <sup>a</sup>	106	0	0
Distilled water <sup>b,*</sup>		316	0	0
Ethanol - 10% <sup>c,*</sup>		96	0	0
Methymethane sulfonated <sup>d,**</sup> - 0.5 $\mu$ m		175	56 <sup>†</sup>	32

LEGEND:

\*Negative control

\*\*Positive control

<sup>†</sup>Significant Difference at  $\leq 0.05$

a. 1.5 ml of ethanol diluted with 15 ml of distilled water and added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic vials.

b. 15.0 ml of distilled water added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

c. 1.5 ml of ethanol diluted to 15.0 ml with distilled water and added to 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

d. 0.5  $\mu$ m of methymethane sulfonate mixed with 5.0 gm of dry food medium (Carolina Biological Formula 4-24). Contents put into plastic exposure vials.

Table J-4. Mutagenic Effects in Drosophila Melanogaster After Exposure by the Inhalation Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke/Exhaust

Exposure substance	Average total hydrocarbon concentration in exposure chamber (mg/l)	Duration of exposure (min)	No. of daily exposures	Exposure container description	X-Chromosomes tested (no.)	Lethal (no.)	(%)
M60A1 Tank-Generated DF2	2.340 ± 0.450	60	5	Glass tube, (35 mm dia x 200 mm long)	206	0	0
Diesel Fuel Smoke/Exhaust	(2,340 ± 450 mg/cu m)			33 mm dia. screen capped one end			
		60	4	Wire screen cylinder (22 mm dia. x 160 mm long)	207	0	0



APPENDIX K

ANALYSES OF ORGAN AND BODY WEIGHTS IN B6C3F1 MICE AND FISCHER 344 RATS  
AFTER AIRBORNE EXPOSURE TO DF2 (DIESEL FUEL) SMOKE AND/OR  
EXHAUST GENERATED BY THE M60A1 TANK

Blank

Table K-1. Organ and Body Weight Analyses in B6C3F<sub>1</sub> Mice Exposed by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> for 65 Daily Exposures and Held 30 Days Post-Exposure

DOSE	SEX	NO.	BODY WEIGHT*		HEART WEIGHT*		LUNG WEIGHT*		LIVER WEIGHT*		KIDNEY WEIGHT*		GONAD WEIGHT*	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	M	6	30.91	1.92	0.20	0.02	0.35	0.03	1.65	0.15	0.65	0.07	0.27	0.03
15 Min Exhaust	M	6	30.37	1.42	0.20	0.024	0.36	0.06	1.79	0.10	0.70	0.07	0.28	0.05
1 Hour Exhaust	M	6	29.90	1.88	0.20	0.05	0.41	0.06	1.70	0.11	0.69	0.09	0.31	0.04
15 Min Smoke/exhaust	M	6	30.61	0.88	0.20	0.02	0.35	0.04	1.68	0.15	0.64	0.06	0.26	0.01
1 Hour Smoke/exhaust	M	6	30.80	2.30	0.22	0.05	0.33	0.05	1.73	0.20	0.71	0.10	0.28	0.05

<sup>a</sup> Average total hydrocarbon concentration of DF2 Smoke/Exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup> Average total hydrocarbon concentration of DF2 Exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\* Statistical analysis is with the Student's "t" test ( $P = < 0.05$ ).

Table K-2. Organ and Body Weight Analyses in B6C3F<sub>1</sub> Mice Exposed by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> for 65 Daily Exposures and Held 30 Days Post-Exposure.

DOSE	SEX	NO.	BODY WEIGHT*		HEART WEIGHT*		LUNG WEIGHT*		LIVER WEIGHT*		KIDNEY WEIGHT*		GONAD WEIGHT *	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	F	6	26.48	1.35	0.17	0.05	0.32	0.05	1.47	0.14	0.45	0.06	N/A	
15 Min Exhaust	F	6	24.84	1.68	0.19	0.05	0.32	0.05	1.40	0.16	0.45	0.07	N/A	
1 Hour Exhaust	F	6	26.44	1.77	0.18	0.04	0.31	0.06	1.52	0.18	0.44	0.06	N/A	
15 Min Smoke / exhaust	F	6	25.85	1.57	0.18	0.03	0.35	0.03	1.53	0.08	0.45	0.02	N/A	
1 Hour Smoke / exhaust	F	6	26.85	0.97	0.19	0.05	0.38	0.02	1.66	0.13	0.51	0.05	N/A	

<sup>a</sup> Average total hydrocarbon concentration of DF2 Smoke/Exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup> Average total hydrocarbon concentration of DF2 Exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\* Statistical analysis is with the Student's "t" test ( $P \leq 0.05$ ).

Table K-3. Organ and Body Weight Analyses in Fischer 344 Rats Exposed by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> for 65 Daily Exposures and held 30 Days Post-Exposure.

DOSE	SEX	NO.	BODY WEIGHT*		HEART WEIGHT*		LUNG WEIGHT*		LIVER WEIGHT*		KIDNEY WEIGHT*		GONAD WEIGHT*	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	M	6	413.83	41.03	1.23	0.16	2.25	0.33	13.63	1.59	2.73	0.39	3.19	0.22
15 Min Exhaust	M	6	414.60	27.40	1.18	0.17	2.38	0.18	14.82	1.84	2.89	0.22	3.33	0.16
1 Hour Exhaust	M	6	394.50	42.76	1.16	0.15	2.47	0.30	13.77	1.45	2.73	0.24	3.28	0.15
15 Min Smoke/exhaust	M	6	403.33	36.76	1.05	0.12	2.33	0.29	13.70	1.92	2.71	0.39	3.19	0.14
1 Hour Smoke/exhaust	M	6	413.33	27.85	1.21	0.20	2.46	0.21	14.48	0.95	2.73	0.20	3.31	0.15

<sup>a</sup> Average total hydrocarbon concentration of DF2 Smoke/Exhaust =  $2.340 \pm 0.450$  mg/l ( $2,340 \pm 450$  mg/cu m)

<sup>b</sup> Average total hydrocarbon concentration of DF2 Exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\* Statistical analysis is with the Student's "t" test ( $P = 40.05$ ).

Table K-4. Organ and Body Weight Analyses in Fischer 344 Rats Exposed by the Airborne Route to M60A1 Tank-Generated DF2 (Diesel Fuel) Smoke<sup>a</sup> and/or Exhaust<sup>b</sup> for 65 Daily Exposures and Held 30 Days Post-Exposure.

DOSE	SEX	NO.	BODY WEIGHT*		HEART WEIGHT*		LUNG WEIGHT*		LIVER WEIGHT*		KIDNEY WEIGHT*		GONAD WEIGHT*	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	F	6	212.33	12.09	0.81	0.20	1.49	0.17	7.14	0.48	1.46	0.14	N/A	
15 Min Exhaust	F	6	210.50	18.29	0.64	0.10	1.48	0.15	7.10	0.89	1.44	0.17	N/A	
1 Hour Exhaust	F	6	207.33	16.49	0.66	0.10	1.45	0.13	6.83	0.64	1.52	0.18	N/A	
15 Min Smoke/exhaust	F	6	219.50	14.39	0.73	0.09	1.60	0.22	7.36	0.70	1.59	0.10	N/A	
1 Hour Smoke/exhaust	F	6	204.67	13.43	0.65*	0.08	1.42	0.09	6.84	0.56	1.40	0.10	N/A	

a Average total hydrocarbon concentration of DF2 Smoke/Exhaust =  $2.340 \pm 0.450$  mg/l ( $2.340 \pm 450$  mg/cu m)

b Average total hydrocarbon concentration of DF2 Exhaust =  $0.006 \pm 0.006$  mg/l ( $6.0 \pm 6.0$  mg/cu m)

\* Statistical analysis is with the Student's "t" test ( $P = \leq 0.05$ ).

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